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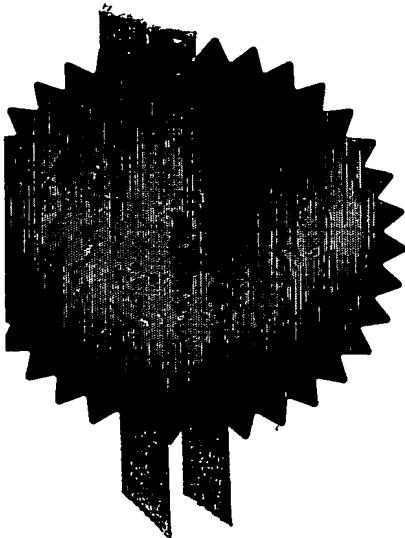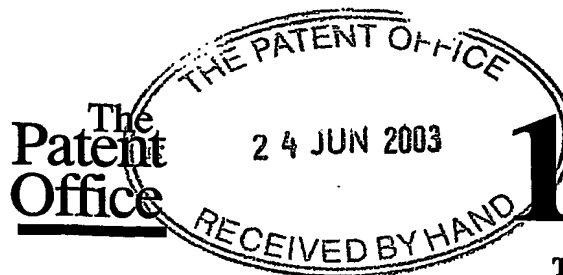
Signed *R. Mahoney*

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The Patent Office
Cardiff Road
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1.	**Your reference**	61.81204

2.	**Patent application number** *(The Patent Office will fill in this part)*	2 4 JUN 2003 25JUN03 E817579-2 D00027 P01/7700 0.00-0314743.6

3.	**Full name, address and postcode of the or of each applicant** *(underline all surnames)*	Isis Innovation Limited Ewert House Ewert Place Summertown Oxford OX2 7SG United Kingdom 0314743.6
	Patents ADP number *(if you know it)*	
	If the applicant is a corporate body, give country/state of incorporation	United Kingdom 3 99 856400

4.	**Title of the invention**	Reagents and methods

5.	**Name of your agent** *(if you have one)*	Frank B. Dehn & Co.
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	179 Queen Victoria Street London EC4V 4EL
	Patents ADP number *(if you know it)*	166001

		Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number			

		Number of earlier application		Date of filing *(day / month / year)*
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application			

8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* a) *any applicant named in part 3 is not an inventor, or* b) *there is an inventor who is not named as an applicant, or* c) *any named applicant is a corporate body.* *See note (d))*	Yes

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Priority documents

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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*

Request for preliminary examination and search *(Patents Form 9/77)*

Request for substantive examination *(Patents Form 10/77)*

Any other documents *(please specify)*

11.

I/We request the grant of a patent on the basis of this application.

frank B. Dehn + Co.

Signature   Date 24 June 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

Annabel R. Beacham
01273 244200

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Reagents and Methods

The present application is concerned with reagents and methods for the formation of disulfide bonds, in particular reagents and methods for use in the glycosylation of proteins.

The glycosylation of proteins plays a vital role in their biological behaviour and stability (R. Dwek, Chem. Rev., 96:683-720 (1996)). Controlling the degree and nature of glycosylation of a protein therefore allows the possibility of investigating and controlling its behaviour in biological systems. A number of methods for the glycosylation of proteins are known, including chemical synthesis. Chemical synthesis of glycoproteins offers certain advantages, not least the possibility of access to pure glycoprotein glycoforms. One known synthetic method utilises thiol-selective carbohydrate reagents, glycosylmethane thiosulfonate reagents (glyco-MTS). Such glycosylmethane thiosulfonate reagents react with thiol groups in a protein to introduce a glycosyl residue linked to the protein via a disulfide bond (see for example WO00/01712).

However, glyco-MTS reagents suffer from a number of disadvantages, including occasionally moderate reaction yields, difficulties in their preparation and problems with stability under the basic conditions in which they are often used. There is therefore a need for further reagents for use in protein glycosylation which are readily prepared, stable and give high yields of the glycosylated protein product.

We have now surprisingly found that certain sulfur and selenium-containing glycosylation reagents are relatively straightforward to prepare, are generally more stable than the corresponding glycol-MTS reagents and can be used in the glycosylation of a wide range of thiol containing compounds, including proteins, in high yield.

In one aspect, the application therefore provides a method of forming disulfide bonds, the method comprising reacting an organic compound comprising at least one thiol group with a reagent of formula I:

$$R-S-X-R^1 \qquad \text{I}$$
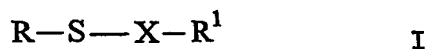
wherein:

X denotes SO_2 or Se;

R denotes an organic moiety, for example an alkyl group, an alkenyl group, an alkynyl group, or a carbohydrate moiety; and

R^1 denotes an optionally substituted alkyl group, an optionally substituted phenyl group, an optionally substituted pyridyl group or an optionally substituted naphthyl group;

with the proviso that when X denotes SO_2 then R^1 does not denote optionally substituted alkyl.

Preferably, the organic compound comprising at least one thiol group is an amino acid, peptide or protein.

The invention further provides a method of chemically modifying a protein, peptide or amino acid comprising at least one thiol group, the method comprising reacting said protein, peptide or amino acid with a compound of formula I as previously defined.

In a still further aspect, the invention provides compounds of formula I wherein R denotes a carbohydrate moiety.

When R denotes an alkenyl or alkenyl group, there is the possibility that the disulphide compound formed by reaction with the compound of formula I may be further elaborated by reaction at the C=C or C≣C bond in the group R.

A generalised reaction scheme for disulfide bond formation is shown in Scheme 1:
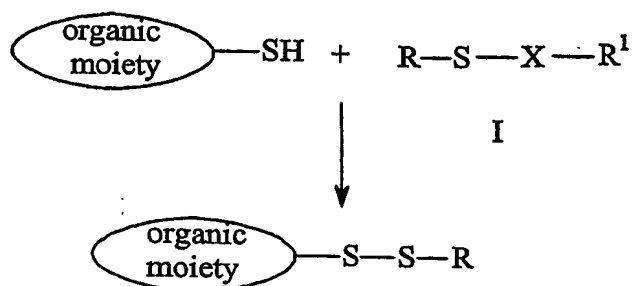
Scheme 1

As used herein, alkyl preferably denotes a straight chain or branched alkyl group containing 1-10 carbon atoms, preferably 1-6 carbon atoms. Preferred alkyl groups include methyl and ethyl. As used herein, alkenyl preferably denotes a straight chain or branched hydrocarbon group comprising at least one carbon-carbon double bond, and containing 2-10 carbon atoms, preferably 2-6 carbon atoms. Preferred alkenyl groups include $-(CH_2)CH=CH_2$ and $-CH_2CH_2CH=CH_2$. As used herein, alkynyl preferably denotes a straight chain or branched hydrocarbon group comprising at least one carbon-carbon triple bond, and containing 2-10 carbon atoms, preferably 2-6 carbon atoms. Preferred alkynyl groups include $-CH_2C{\equiv}CH$ and $-CH_2CH_2C{\equiv}CH$.

When R^1 denotes an optionally substituted moiety, suitable substituents include any substituents which do not interfere with the formation of the compound of formula I or with the disulfide bond forming reaction, for example $-NO_2$, $-SO_3H$, $-CO_2H$, and $-(CH_2CH_2O)_nH$ wherein n denotes 1-100, preferably 1-50, more preferably 1-20, and still more preferably 1-10. The R^1 group may be independently substituted by 1-5, and preferably 1 or 2, substituents.

A preferred R^1 group is phenyl. When the group R^1 in the compounds of formula I is phenyl or another aromatic group, then there is the added advantage that the progress of the reaction with the thiol-containing compound may be monitored using UV spectroscopy. Thus, for example, the $PhSO_2-$ chromophore displays a maximum in

the UV spectrum at approx. 265nm. The $PhSO_2$- moiety is
present in both the compound of formula I and the $PhSO_2^-$
that is the by-product of the disulfide bond forming
reaction, but the associated extinction coefficients
differ sufficiently for the progress of the reaction to
be monitored using UV.

In the compounds of formula I, the group R may be
any organic moiety but is preferably a carbohydrate
moiety, optionally attached via a linker to the -S-X-
group. The linker may contain 1 to 10 atoms between the
carbohydrate moiety and the -S-X- group. For example,
the linker may be an alkylene group (for example a
-$(CH_2)_t$- group wherein t denotes 1 to 10), or an
alkenylene group (for example a -(CH_2)CH=CH- or
-CH_2CH_2CH=CH- group).

Suitable carbohydrate moieties include
monosaccharides, oligosaccharides and polysaccharides,
and include any carbohydrate moiety which is present in
naturally occurring glycoproteins. Preferred are
optionally protected glycosyl or glycoside derivatives,
for example optionally-protected glucosyl, glucoside,
galactosyl or galactoside derivatives. Glycosyl and
glycoside groups include both α and β groups. Suitable
carbohydrate moieties include glucose, galactose,
fucose, GlcNAc, GalNAc, sialic acid, and mannose, and
oligosaccharides or polysaccharides comprising at least
one glucose, galactose, fucose, GlcNAc, GalNAc, sialic
acid, and/or mannose residue.

Any functional groups in the carbohydrate moiety
may optionally be protected using protecting groups
known in the art (see for example Greene et al,
"Protecting groups in organic synthesis", 2nd Edition,
Wiley, New York, 1991, the disclosure of which is hereby
incorporated by reference). Suitable protecting groups
for any -OH groups in the carbohydrate moiety include
acetate (Ac), benzyl (Bn), silyl (for example tert-butyl
dimethylsilyl (TBDMSi) and tert-butyldiphenylsilyl
(TMDPSi)), acetals, ketals, and methoxymethyl (MOM).
Any protecting groups may be removed before or after

attachment of the carbohydrate moiety to the amino acid, peptide or protein.

Particularly preferred carbohydrate moieties include $Glc(Ac)_4\beta-$, $Glc(Bn)_4\beta-$, $Gal(Ac)_4\beta-$, $Gal(Bn)_4\beta-$, $Glc(Ac)_4\alpha(1,4)Glc(Ac)_3\alpha(1,4)Glc(Ac)_4\beta-$, $\beta-Glc$, $\beta-Gal$, $-Et-\beta-Gal$, $-Et-\beta-Glc$, $Et-\alpha-Glc$, $-Et-\alpha-Man$, $-Et-Lac$, $-\beta-Glc(Ac)_2$, $-\beta-Glc(Ac)_3$, $-Et-\alpha-Glc(Ac)_2$, $-Et-\alpha-Glc(Ac)_3$, $-Et-\alpha-Glc(Ac)_4$, $-Et-\beta-Glc(Ac)_2$, $-Et-\beta-Glc(Ac)_3$, $-Et-\beta-Glc(Ac)_4$, $-Et-\alpha-Man(Ac)_3$, $-Et-\alpha-Man(Ac)_4$, $-Et-\beta-Gal(Ac)_3$, $-Et-\beta-Gal(Ac)_4$, $-Et-Lac(Ac)_5$, $-Et-Lac(Ac)_6$, $-Et-Lac(Ac)_7$, and their deprotected equivalents.

Preferably, any saccharide units making up the carbohydrate moiety which are derived from naturally occurring sugars will each be in the naturally occurring enantiomeric form, normally the D-form. Any anomeric linkages may be $\alpha-$ or $\beta-$ linkages.

The compound comprising a thiol group may be any organic compound which comprises at least one thiol group. The thiol group may be primary, secondary or tertiary. The compound may be aromatic or aliphatic. If more than one thiol group is present in the compound, a disulfide bond will potentially be formed at each such thiol group.

Preferably, the compound is an amino acid, a peptide or a protein. Any amino acid is preferably an α-amino acid. It may optionally be incorporated into a peptide or protein. Any amino acid may be in the D- or L-form, preferably the L-form. The amino acid, peptide or protein may be any naturally-occurring amino acid, peptide or protein which comprises a thiol group, for example due to the presence of one or more cysteine residues. Alternatively, the amino acid, peptide or protein may be prepared by chemical modification of a precursor non-thiol containing amino acid, peptide or protein. Alternatively, a thiol containing peptide or protein may be prepared via site-directed mutagenesis to introduce a cysteine residue. Site-directed mutagenesis

is a known technique in the art (see for example WO00/01712 and J. Sambrook *et al*, Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Springs Harbour Laboratory Press, 2001, the disclosures of which are hereby incorporated by reference).

Preferred proteins include enzymes, the selectivity of which may be modified by controlled glycosylation using the methods and reagents according to the invention. Other preferred proteins include serum albumins and other blood proteins, hormones, interferons, receptors, antibodies, and interleukins.

It has been found that the compounds of formula I are normally thiol-selective, and hence that the presence of other functional groups in the thiol-containing organic compound does not normally interfere with the reaction. However, any other functional groups may optionally be protected using any protecting groups known in the art which are stable under the reaction conditions.

The disulfide bond forming reaction is generally carried out in the presence of a buffer at neutral or basic pH (pH 7 to 9.5), with slightly basic pHs being preferred (pH 8 to 9). Suitable buffers include HEPES, CHES, MES and Tris. If the thiol-containing compound is a protein, peptide or amino acid, the pH should be such that little or no unwanted denaturation occurs during the reaction. Similarly, the reaction temperature should be selected to avoid any significant damage to any temperature sensitive compounds. For example, a reaction with a protein or peptide is preferably carried out at ambient temperature or below to avoid any denaturation. Aqueous or organic solvent systems may be used, with aqueous solvent systems being preferred for the reaction of proteins, amino acids or peptides to ensure their dissolution. The reaction is generally fairly quick, for example often taking less than 1 hour.

In general, an excess of the compound of formula I will be used, for example 10-20 equivalents based on the

thiol-containing compound. In contrast, reactions with glyco-MTS reagents often require the use of approximately 30 equivalents, adding to the cost of the reagents.

It has been found that the compounds of formula I wherein R denotes a carbohydrate moiety, X denotes SO_2 and R^1 denotes phenyl are generally more stable to basic conditions than the corresponding glyco-MTS compounds. Any unreacted or excess compound of formula I may therefore often be recovered from the reaction for reuse, which is particularly advantageous when R denotes a carbohydrate moiety as such compounds may be relatively expensive and/or time consuming to prepare. Furthermore, the phenyl thiosulfonate compounds of formula I are generally cheaper and easier to prepare than the corresponding MTS compounds.

The compounds of formula I may be prepared by a number of different methods. Compounds wherein X denotes SO_2 maybe prepared by reacting a compound of formula II:

$$M(SSO_2R^1)_k \qquad\qquad II$$

wherein:

M denotes a metal, for example Li, Na, K, Cs, Ca, Mg, Zn, or Al, preferably Na or K; and
k denotes 1, 2 or 3;

with a compound of formula III:

$$R-L \qquad\qquad III$$

wherein:

R is as defined for the compounds of formula I and L denotes a leaving group.

Any leaving group L may be utilised as long as the resultant anion L^- does not interfere with the reaction in any way, for example by reacting with the product.

Preferred leaving groups L include halo and sulfonates such as toluenesulfonate (tosylate), methanesulfonate (mesylate) and trifluoromethane sulfonate (triflate), in particular chloro and bromo.

Compounds of formula III are commercially available or may be prepared using methods known in the art, for example methods for the formation of halo-sugars in general and 1-halo-sugars in particular. Preferably the compound of formula III is a glycosyl halide. Examples of suitable compounds of formula III based on glucose and galactose are shown generically below:
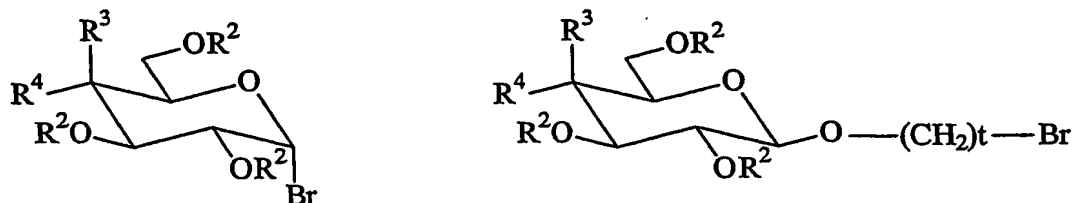
wherein:

each R^2 independently denotes H, or a suitable protecting group for example Ac or Bn;

one of R^3 and R^4 denotes H and the other denotes OH, O-protecting group or O-saccharide moiety; and

t denotes 1 to 10, preferably 1 to 6, more preferably 1 or 2.

The reaction may be carried out in any solvent-system in which the compound of formula III is soluble. Preferably, the compound of formula II is also at least partially soluble in the solvent system. Suitable solvents include alkanols such as ethanol and methanol, N,N-dimethylformamide (DMF) and acetonitrile, with acetonitrile being particularly preferred.

The compounds of formula II may be prepared by reacting the corresponding sulfinite salt (formula VII) with sulfur, as shown in Scheme 2:

$$M(SO_2R^1)_k \ + \ S \ \rightarrow \ M(SSO_2R^1)_k$$

VII   II
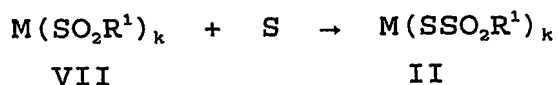
Scheme 2

Compounds of formula II which are crystalline are preferred for ease of purification, especially on a large scale.

Sulfinite salts of formula VII are available commercially (for example sodium benzenesulfinite) or may be prepared by methods known in the art (see for example JP 61205249, and M. Uchino et al, Chemical & Pharmaceutical Bulletin, 1978, 26(6), 1837-45, the disclosures of which are hereby incorporated by reference). For example, the corresponding thiolate salt R^1S- may be prepared by deprotonation of the corresponding thiol compound R^1SH using a suitable base, for example methyl lithium. The thiolate salt may then be oxidised to the corresponding sulfinite salt using a suitable oxidising agent, for example 2-(phenylsulfonyl)-3-phenyloxaziridine (the "Davis reagent") (Sandrinelli et al, Organic Letters (1999), 1(8), 1177-1180, the disclosure of which is hereby incorporated by reference).

Alternatively, compounds of formula I in which X denotes SO_2 may be prepared by reacting a disulfide of formula VIII with a sulfinite anion $R^1SO_2^-$ in the presence of silver ions, as shown in Scheme 3:

$$R-\overset{+}{\underset{\underset{Ag}{|}}{S}}-S-R \ + \ R^1SO_2^- \ \longrightarrow \ R-S-SO_2-R^1$$
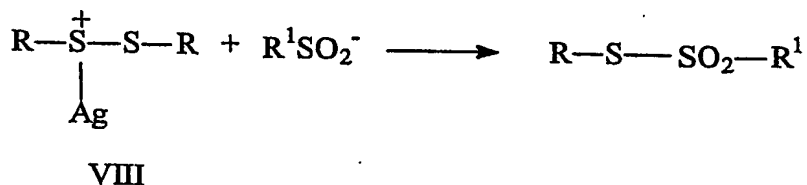
VIII

Scheme 3

Disulfide compounds of formula VIII are commercially available or may be prepared using methods known in the art.

Compounds of formula I wherein X denotes Se may be formed by reaction of a compound of formula V:

$$R\text{---}SH \qquad\qquad V$$

wherein R is as defined for the compounds of formula I, with a compound of formula VI:

$$R^1SeL^2 \qquad\qquad VI$$

wherein R^1 is as defined for the compounds of formula I, and L^2 denotes Br, Cl, CN, or I. Alternatively, $PhSe(OH)_2$ may be used instead of the compound of formula VI. The reaction may be carried out in anhydrous dichloromethane and then quenched by the addition of triethylamine.

The compounds of formula VI are commercially available (e.g. PhSeBr, PhSeCl) or may be prepared by methods known in the art. For example, MeSeBr may be prepared according to the method of Hope, Eric G.; Kemmitt, Tim; and Levason, William, in Journal of the Chemical Society, Perkin Transactions 2: Physical Organic Chemistry (1972-1999) (1987), (4), 487-90, the disclosure of which is hereby incorporated by reference.

The compounds of formula V are commercially available or may be prepared by methods known in the art for the preparation of thiol compounds in general, and thio-sugars in particular. When R in the compound of formula V denotes a carbohydrate moiety, the thiol group may be at any position in the moiety. Preferably, it is at the anomeric position of a saccharide or is attached to the anomeric carbon via a linker. Examples of suitable compounds of formula III based on glucose and galactose are shown generically below:
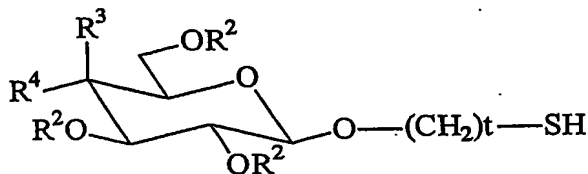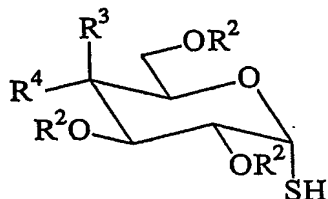
wherein:

each R² independently denotes H, or a suitable protecting group, for example Ac or Bn;
one of R³ and R⁴ denotes H and the other denotes OH, O-protecting group or O-saccharide moiety; and
t denotes 1 to 10, preferably 1 to 6, more preferably 1 or 2.

In the reaction of the compounds of formula V with the compounds of formula VI, any other functional groups in the compound of formula V may be unprotected, or may be protected by protecting groups known in the art.

The invention will be further illustrated by the following non-limiting Examples.

Example 1: Sodium phenylthiosulfonate (NaPTS)
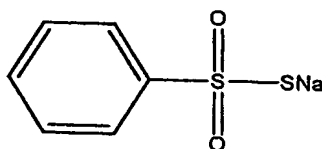
Sodium benzenesulfinate (10 g, 61 mmol) and sulfur (1.95 g, 61 mmol) were dissolved in anhydrous pyridine (60 mL) to give a yellow solution. The reaction was stirred under argon and after 1 h gave a white suspension. The reaction was filtered and washed with anhydrous diethyl ether. Recrystallisation from anhydrous ethanol afforded the title product (10.5 g, 88%) as a white crystalline solid; m.p. 305-306°C [Lit. 287°C, Sato, R.; Goto, T.; Takikawa, Y.; Takizawa, S. *Synthesis* 1980, 615]; δ_H (200 MHz, DMSO-d_6) 7.28-7.76 (5H, m, Ar-H).

Example 2: 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl phenylthiosulfonate
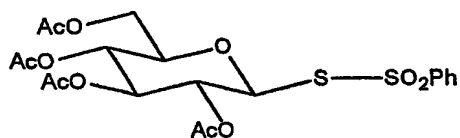
2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide
(207 mg, 0.5 mmol) was dissolved in anhydrous
acetonitrile (5 mL). To this sodium phenylthiosulfonate
(201 mg, 1 mmol) and tetrabutylammonium bromide (16 mg,
0.05 mmol) were added. The resulting mixture was stirred
under argon at 70°C. After a 4.5 h period, thin layer
chromatography (t.l.c.) (petrol:ethyl acetate, 1:1)
indicated the formation of a product (R_f 0.5) with
complete consumption of the starting material (R_f 0.3).
The solution was concentrated *in vacuo*. The crude solid
was partitioned between dichloromethane (DCM, 20 mL) and
water (20 mL), and the aqueous layer re-extracted with
DCM (2 x 20 mL). The combined organics were washed with
brine (20 mL), dried over $MgSO_4$, filtered and
concentrated *in vacuo*. The residue was purified by flash
column chromatography (petrol:ethyl acetate, 1:1) to
afford the title product (225 mg, 88%) as a white
crystalline solid; mp 129-130°C; $[\alpha]_D^{25}$ +51.2 (c, 1.0 in
CHCl$_3$); υ_{max} (KBr) 1754 (s, C=O), 1376 (s, C=C) cm^{-1}; δ_H (400
MHz, C_6D_6) 1.68, 1.72, 1.73, 1.75 (4 x 3H, 4 x s,
4 x OAc), 3.09 (1H, ddd, $J_{4,5}$ 10.2 Hz, $J_{5,6}$ 2.4 Hz, $J_{5,6'}$
4.2 Hz, H-5), 3.83 (1H, dd, $J_{5,6}$ 2.4 Hz, $J_{6,6'}$ 12.7 Hz,
H-6), 4.08 (1H, dd, $J_{5,6'}$ 4.2 Hz, $J_{6,6'}$ 12.6 Hz, H-6'),
5.17-5.23 (2H, m, H-2, H-4), 5.40 (1H, d, $J_{1,2}$ 10.2 Hz,
H-1), 5.44 (1H, at, J 9.4 Hz, H-3), 6.98-7.03 (3H, m,
Ar-H), 7.90-7.92 (2H, m, Ar-H). The structure of the
product was further confirmed by single crystal X-ray
diffraction.

Example 3: 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl
phenylthiosulfonate
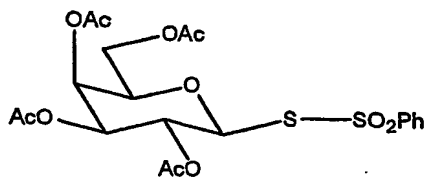
2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (2.0 g, 5 mmol) was dissolved in anhydrous acetonitrile (80 mL). To this sodium phenylthiosulfonate (2.02 g, 10.3 mmol) and tetrabutylammonium bromide (160 mg, 0.5 mmol) were added. The resulting mixture was stirred under argon at 70°C. After a 5 h period, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a product (R_f 0.4) with complete consumption of the starting material (R_f 0.6). The solution was concentrated *in vacuo*. The crude oil was partitioned between DCM (50 mL) and water (50 mL), and the aqueous layer re-extracted with DCM (2 x 50 mL). The combined organics were washed with brine (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 2:1) to afford the title product (1.7 g, 65%, 2 steps) as a white crystalline solid; mp 53-54°C; $[\alpha]_D^{27}$ +24.2 (c, 1.0 in $CHCl_3$); ν_{max} (KBr) 1756 (s, C=O), 1366 (s, C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.98, 2.03, 2.06, 2.11 (4 x 3H, 4 x s, 4 x OAc), 3.85 (1H, dd, $J_{5,6}$ 8.8 Hz, $J_{6,6'}$ 14.0 Hz, H-6), 3.95-4.00 (2H, m, H-5, H-6), 5.11 (1H, dd, $J_{2,3}$ 9.7 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.23 (1H, at, J 10.3 Hz, H-2), 5.25 (1H, d, $J_{1,2}$ 10.2 Hz, H-1), 5.43 (1H, dd, $J_{3,4}$ 3.6 Hz, $J_{4,5}$ 1.0 Hz, H-4), 7.54-7.68 (3H, m, Ar-H), 7.93-7.97 (2H, m, Ar-H).
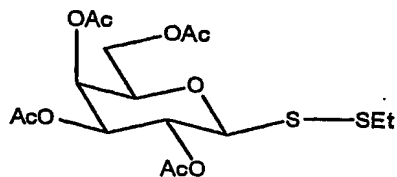
Example 4: Ethyl 2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyranosyl disulfide
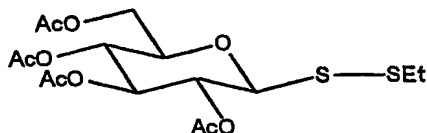
2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl

phenylthiosulfonate (100 mg, 0.2 mmol) and triethylamine
(0.03 mL, 0.2 mmol) were dissolved in anhydrous DCM
(10 mL) and stirred at room temperature (RT) under an
atmosphere of argon. A solution of ethane thiol
(0.016 mL, 0.2 mmol) in anhydrous DCM (10 mL) was slowly
added dropwise *via* a syringe pump over a 30 min period.

After a 40 min period, t.l.c. (petrol:ethyl acetate,
1:1) indicated the formation of a major product (R_f 0.5)
along with complete consumption of the starting material
(R_f 0.3). The solution was concentrated *in vacuo*. The
residue was purified by flash column chromatography
(petrol:ethyl acetate, 1:1) to afford the title product
(70 mg, 82%) as a white crystalline solid; mp 95-96°C
[Lit. 100-102°C, (Davis, B. G.; Ward, S. J.; Rendle, P.
M. *Chem. Commun.* 2001, 189)]; $[\alpha]_D^{22}$ -164.9 (c, 0.2 in

CHCl$_3$) [Lit. $[\alpha]_D^{24}$ -178.0 (c, 1.0 in MeOH) (Davis, B. G.;
Ward, S. J.; Rendle, P. M. *Chem. Commun.* 2001, 189)]; δ_H
(400 MHz, CDCl$_3$) 1.30 (1H, t, *J* 7.4 Hz, CH$_3$), 2.00, 2.02,
2.03, 2.06 (4 x 3H, 4 x s, 4 x CH$_3$), 2.79 (2H, dq, J_{CH3-H}
7.5 Hz, J_{HH} 2.7 Hz), 3.73 (1H, ddd, $J_{4,5}$ 10.2 Hz,
$J_{5,6}$ 2.5 Hz, $J_{5,6'}$ 4.8 Hz, H-5), 4.14 (1H, dd, $J_{5,6}$ 2.4 Hz,
$J_{6,6'}$ 12.4 Hz, H-6), 4.22 (1H, dd, $J_{5,6'}$ 4.7 Hz, $J_{6,6'}$
12.4 Hz, H-6'), 4.52 (1H, d, $J_{1,2}$ 9.8 Hz, H-1), 5.10 (1H,
at, *J* 9.8 Hz, H-4), 5.21-5.26 (2H, m, H-2, H-3).

Example 5: Ethyl 2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-
galactopyranosyl disulfide

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl
phenylthiosulfonate (100 mg, 0.2 mmol) and triethylamine
(0.03 mL, 0.2 mmol) were dissolved in anhydrous DCM
(10 mL) and stirred at RT under an atmosphere of argon.
A solution of ethane thiol (0.016 mL, 0.2 mmol) in
anhydrous DCM (10 mL) was slowly added dropwise *via* a
syringe pump over a 30 min period. After a 40 min
period, t.l.c. (petrol:ethyl acetate, 1:1) indicated the
formation of a major product (R_f 0.4) along with complete
consumption of the starting material (R_f 0.3). The
solution was concentrated *in vacuo*. The residue was
purified by flash column chromatography (petrol:ethyl
acetate, 1:1) to afford the title product (78 mg, 91%)
as a white crystalline solid; mp 65-66°C; $[\alpha]_D^{25}$ -52.1 (c,
1.4 in CHCl$_3$); υ_{max} (KBr) 1746 (s, C=O) cm^{-1}; δ_H (400 MHz,
CDCl$_3$) 1.30 (1H, t, *J* 7.4 Hz, CH$_3$), 1.95, 2.01, 2.02,
2.13 (4 x 3H, 4 x s, 4 x CH$_3$), 2.79 (2H, dq, J_{CH3-H} 7.2 Hz,
J_{HH} 1.7 Hz), 3.94 (1H, td, $J_{4,5}$ 0.9 Hz, $J_{5,6}$ 6.3 Hz,
$J_{5,6'}$ 7.0 Hz, H-5), 4.06 (1H, dd, $J_{5,6}$ 6.3 Hz, $J_{6,6'}$ 11.3 Hz,
H-6), 4.12 (1H, dd, $J_{5,6'}$ 7.0 Hz, $J_{6,6'}$ 11.2 Hz, H-6'), 4.51
(1H, d, $J_{1,2}$ 9.9 Hz, H-1), 5.05 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$
3.6 Hz, H-3), 5.35-5.40 (2H, m, H-2, H-4).

Example 6: *bis-N*-Acetyl-L-cysteinyl-L-serine methylester
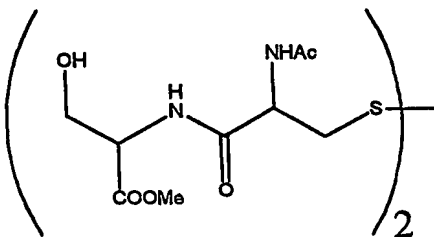
bis-L-Cysteinyl-L-serine methylester (100 mg, 0.23 mmol) was dissolved in methanol (5 mL). To this solution acetic anhydride (0.09 mL, 0.92 mmol) and pyridine (0.075 mL, 0.92 mmol) were added. After a 15 min period, t.l.c. (ethyl acetate:methanol 5:1) indicated the formation of a major product (R_f 0.5) along with complete consumption of the starting material (R_f 0.1). The reaction was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate:methanol 5:1) to afford the title product (60 mg, 50%) as a white crystalline solid; mp 145-147°C; $[\alpha]_D^{25}$ -33.4 (c, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.04 (3H, s, $COCH_3$), 2.96 (1H, dd, $J_{CH,H}$ 13.9 Hz, $J_{CH,H}$ 4.7 Hz, CysC$\underline{H}$H), 3.23 (1H, dd, $J_{CH,H}$ 13.9 Hz, $J_{CH,\alpha H}$ 4.7 Hz, CysCH$\underline{H}$), 3.76 (3H, s, OMe), 3.83 (1H, dd, $J_{CH,H}$ 11.4 Hz, $J_{CH,\alpha H}$ 4.1 Hz, SerC$\underline{H}$H), 3.93 (1H, dd, $J_{CH,H}$ 11.3 Hz, $J_{CH,\alpha H}$ 4.9 Hz, SerCH$\underline{H}$), 4.55 (1H, t, J 4.3 Hz, αHSer), 4.87 (1H, t, J 4.8, αHCys).
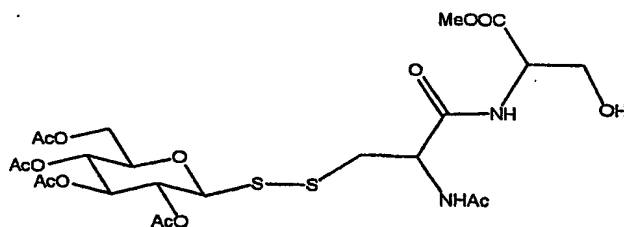
Example 7: *N*-Acetyl-L-cysteinyl-L-serine methylester
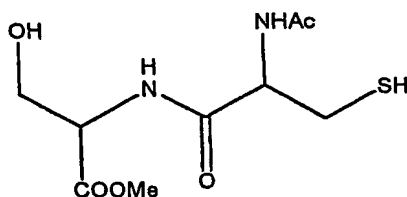
bis-*N*-Acetyl-L-cysteinyl-L-serine methylester (1.92 g, 3.96 mmol) was dissolved in wet chloroform (100 mL) and methanol (10 mL) and stirred. To this stirred solution

tributylphosphine (1.1 mL, 4.36 mmol) was added. After a 2 h period, t.l.c. (ethyl acetate:methanol 10:1) indicated the formation of a product (R_f 0.6) along with complete consumption of the starting material (R_f 0.3). The reaction was concentrated *in vacuo*. Recrystallisation from ethyl acetate/methanol afforded the title product (1.77 g, 93%) as a white crystalline solid; mp 127-128°C; $[\alpha]_D^{25}$ -32.0 (c, 1.0 in MeOH); δ_H (400 MHz, $CDCl_3$) 1.89 (1H, at, J 8.9 Hz, SH), 2.06 (3H, s, $COCH_3$), 2.84-2.93 (1H, m, CysC$\underline{H}$H), 2.97-3.04 (1H, m, CysCH$\underline{H}$), 3.79 (3H, s, OMe), 3.91 (1H, dd, $J_{CH,H}$ 11.4 Hz, $J_{CH,\alpha H}$ 3.1 Hz, SerC$\underline{H}$H), 4.03 (1H, dd, $J_{CH,H}$ 11.7 Hz, $J_{CH,\alpha H}$ 4.2 Hz, SerCH$\underline{H}$), 4.61-4.65 (1H, m, αHSer), 4.71-4.76 (1H, m, αHCys), 6.93 (1H, d, $J_{\alpha H,NH}$ 7.8 Hz, NHCys), 7.73 (1H, d, $J_{\alpha H,NH}$ 7.4 Hz, NHSer).
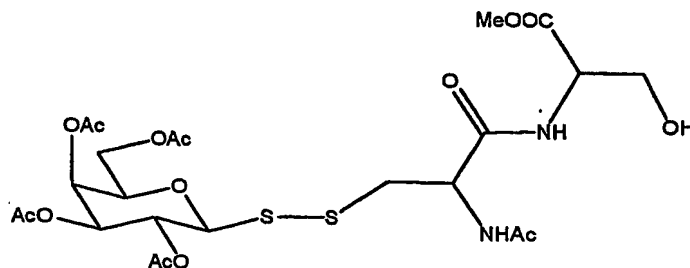
Example 8: *N*-Acetyl-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β- D-glucopyranosyl disulfide)-L-serine methylester

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl phenylthiosulfonate (61 mg, 0.12 mmol) was dissolved in anhydrous DCM (5 mL) and stirred at RT under an atmosphere of argon. To this *N*-acetyl-L-cysteine-L-serine methylester (32 mg, 0.12 mmol) and triethylamine (0.015 mL, 0.11mmol) in anhydrous DCM (10 mL) and anhydrous methanol (0.5 mL) were slowly added dropwise *via* a syringe pump over a 4 h period. After a 5 h

period, t.l.c. (ethyl acetate:methanol, 10:1) indicated the formation of a major product (R_f 0.5) along with complete consumption of the starting material (R_f 0.3, (t.l.c system (petrol:ethyl acetate, 1:1)). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate:methanol, 10:1) to afford the title product (75 mg, 99%) as a white crystalline solid; mp 126-128°C [Lit. 125-128°C (Davis, B. G.; Ward, S. J.; Rendle, P. M. *Chem. Commun.* 2001, 189)]; $[\alpha]_D^{25}$ -47.9 (c, 0.7 in $CHCl_3$) [Lit. $[\alpha]_D^{24}$ - 178.0 (c, 1.0 in MeOH) (Davis, B. G.; Ward, S. J.; Rendle, P. M. *Chem. Commun.* 2001, 189)]; δ_H (400 MHz, $CDCl_3$) 2.03, 2.06, 2.07, 2.11 (5 x 3H, 4 x s, 5 x CH_3), 3.05 (1H, dd, $J_{CH,H}$ 13.9 Hz, $J_{CH,\alpha H}$ 8.8 Hz, CysCHH), 3.28 (1H, dd, $J_{CH,H}$ 13.9 Hz, $J_{CH,\alpha H}$ 4.8 Hz, CysCHH), 3.80 (3H, s, OMe), 3.89 (1H, ddd, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 2.2 Hz, $J_{5,6'}$ 4.1 Hz, H-5), 3.94 (1H, dd, $J_{CH,H}$ 11.7 Hz, $J_{CH,\alpha H}$ 3.0 Hz, SerCHH), 4.00 (1H, dd, $J_{CH,H}$ 13.8 Hz, $J_{CH,\alpha H}$ 3.7 Hz, SerCHH), 4.23 (1H, dd, $J_{5,6}$ 4.2 Hz, $J_{6,6'}$ 12.4 Hz, H-6), 4.38 (1H, dd, $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 12.5 Hz, H-6'), 4.62-4.65 (1H, m, αHSer), 4.64 (1H, d, $J_{1,2}$ 9.5 Hz, H-1), 4.90-4.94 (1H, m, αHCys), 5.18 (1H, at, J 10.1 Hz, H-4), 5.24-5.29 (2H, m, H-2, H-3), 6.94 (1H, d, $J_{NH,H}$ 7.9 Hz, NHAc), 7.52 (1H, d, $J_{NH,H}$ 7.6 Hz, NHSer).

Example 9: *N*-Acetyl-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-galactopyranosyl disulfide)-L-serine methylester

2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl

phenylthiosulfonate (50 mg, 0.1 mmol) was dissolved in anhydrous DCM (5 mL) and stirred at RT under an atmosphere of argon. A solution of N-acetyl-L-cysteine-L-serine methylester (31 mg, 0.12 mmol) and triethylamine (0.015 mL, 0.11mmol) in anhydrous DCM (10 mL) and anhydrous methanol (0.5 mL) was slowly added dropwise via a syringe pump over a 2 h period. After a 2 h period, t.l.c. (ethyl acetate:methanol, 10:1) indicated the formation of a major product (R_f 0.5) along with complete consumption of the starting material (R_f 0.5, t.l.c system petrol:ethyl acetate, 1:1). The solution was concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate:methanol, 10:1) to afford the title product (59 mg, 95%) as a white amorphous solid; $[\alpha]_D^{25}$ -48.8 (c, 0.25 in CHCl$_3$); δ_H (400 MHz, CDCl$_3$) 1.99, 2.04, 2.05, 2.08, 2.18 (5 x 3H, 4 x s, 5 x CH$_3$), 2.80 (1H, bs, OH), 2.99 (1H, dd, $J_{CH,H}$ 14.1 Hz, $J_{CH,\alpha H}$ 9.2 Hz, CysCHH), 3.32, 3.77 (3H, s, OMe), 3.92 (1H, dd, $J_{CH,H}$ 11.7 Hz, $J_{CH,\alpha H}$ 3.0 Hz, SerCHH), 4.01 (1H, dd, $J_{CH,H}$ 11.7 Hz, $J_{CH,\alpha H}$ 3.7 Hz, SerCHH), 4.06-4.14 (2H, m, H-5, H-6), 4.20-4.26 (1H, m, H-6'), 4.61-4.63 (1H, m, αHSer), 4.65 (1H, d, $J_{1,2}$ 9.8 Hz, H-1), 4.88-4.93 (1H, m, αHCys), 5.11 (1H, dd, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.42-5.47 (2H, m, H-2, H-4), 6.68 (1H, d, $J_{NH,H}$ 7.8 Hz, NHAc), 7.28 (1H, d, $J_{NH,H}$ 8.1 Hz, NHSer).

Example 10: 2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl bromide
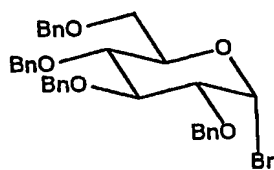
2,3,4,6-Tetra-O-benzyl-D-glucopyranose (1.0 g, 1.9 mmol)
was dissolved in anhydrous DCM (6 mL) and anhydrous DMF
(0.4 mL) under argon. The resulting solution was stirred
at 0°C. Oxalyl bromide (4 mL, 2M in DCM, 24 mmol) was
added dropwise over a 5 min period . The reaction was
stirred at RT. After a 40 min period, t.l.c.
(petrol:ethyl acetate, 2:1) indicated the formation of a
major product (R_f 0.7). The reaction was cooled to 0°C
and quenched with ice cold water (30 mL) added over a 5
min period. The reaction was partitioned between DCM
(20 mL) and water. The aqueous layer was re-extracted
with DCM (3 x 20 mL), the combined organic layers were
washed with brine (40 mL), dried (MgSO$_4$), filtered and
concentrated in vacuo to afford the title product (1.10

g, 95%) as a crude yellow oil; δ_H (400 MHz, CDCl$_3$), 3.57
(1H, dd, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 9.1 Hz, H-2), 3.68 (1H, dd, $J_{5,6}$
2.1 Hz, $J_{6,6'}$ 11.0 Hz, H-6), 3.79-3.84 (2H, m, H-4, H-6'),
4.07 (1H, at, J 9.1 Hz, H-3), 4.07-4.11 (1H, m, H-5),
4.47-4.62 (3H, m, PhCH$_2$), 4.74 (s, 2H, PhCH$_2$), 4.84-4.89
(2H, m, PhCH$_2$), 5.10 (1H, d, J 11.1 Hz, PhCH$_2$), 6.46 (1H,
d, H-1), 7.15-7.41 (20H, m, Ar-H).

Example 11: 2,3,4,6-Tetra-O-benzyl-β-D-glucopyranosyl phenylthiosulfonate
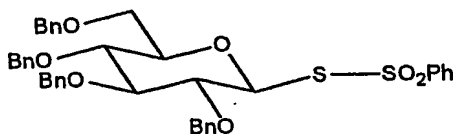
2,3,4,6-Tetra-O-benzyl-D-α-glucopyranosoyl bromide (3.55
g, 5.88 mmol) and sodium phenylthiosulfonate

(4.76 g, 24.3 mmol) were dissolved in anhydrous 1,4 dioxane (90 mL). The reaction was heated to 70°C under argon. After 20 h, t.l.c. (petrol:ethyl acetate, 2:1) indicated the formation of a major product (R_f 0.6) with complete consumption of the starting material (R_f 0.7). The reaction was cooled to RT and filtered, the precipitate was washed with petrol/ethyl acetate and the filtrate concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 4:1) to afford 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl phenylthiosulfonate (3.18 g, 78%) as a white viscous gum as a mixture of α,β compounds both in a β:α ratio of 3:1. Selective re-crystallisation from ethyl acetate/petrol afforded pure 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl phenylthiosulfonate as a white crystalline solid; m.p. 106-108°C; $[\alpha]_D^{22}$ +21.4 (c, 0.35 in $CHCl_3$); δ_H (500 MHz, C_6D_6) 3.21 (1H, ddd, $J_{4,5}$ 9.7 Hz, $J_{5,6}$ 1.4 Hz, $J_{5,6'}$ 3.8 Hz, H-5), 3.29 (1H, dd, $J_{5,6}$ 1.4 Hz, $J_{6,6'}$ 11.1 Hz, H-6), 3.34 (1H, dd, $J_{1,2}$ 9.9 Hz, $J_{2,3}$ 8.7 Hz, H-2), 3.49 (1H, dd, $J_{5,6}$ 3.8 Hz, $J_{6,6'}$ 11.1 Hz, H-6'), 3.51 (1H, at, J 9.4 Hz, H-3), 3.60 (1H, at, J 9.4 Hz, H-4), 4.15, 4.25 (2H, ABq, J 12.1 Hz, $PhCH_2$), 4.52, 4.58 (2H, ABq, J 11.0 Hz, $PhCH_2$), 4.72, 4.76 (2H, ABq, J 11.3 Hz, $PhCH_2$), 4.78, 4.52 (2H, ABq, J 11.3 Hz, $PhCH_2$), 5.25 (1H, d, $J_{1,2}$ 10.2 Hz, H-1), 6.82-6.88 (3H, m, Ar-H), 7.05-7.26 (20H, m, Ar-H), 7.96-7.98 (2H, m, Ar-H).

Example 12: Ethyl 2,3,4,6-tetra-*O*-benzyl-1-dithio-β-D-glucopyranosyl disulfide
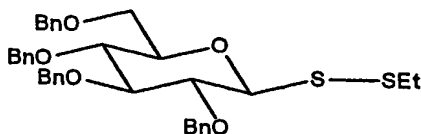
2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl phenylthiosulfonate (100 mg, 0.14 mmol) and

triethylamine (0.02 mL, 0.14 mmol) were dissolved in anhydrous DCM (10 mL) and stirred at RT under an atmosphere of argon. To this ethane thiol (11 µL, 0.14 mmol) in anhydrous DCM (10 mL) was slowly added dropwise *via* a syringe pump over a 90 min period. After a 90 min period, t.l.c. (petrol:ethyl acetate, 6:1) indicated the formation of a major product (R_f 0.4) along with complete consumption of the starting material (R_f 0.2). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 7:1) to afford the title product (83 mg, 95%) as a clear oil; $[\alpha]_D^{22}$ -164.9 (c, 0.2 in CHCl$_3$) [Lit. $[\alpha]_D^{25}$ -80.0 (c, 3.0 in MeOH) (Davis, B. G.; Ward, S. J.; Rendle, P. M. *Chem. Commun.* 2001, 189)]; δ_H (400 MHz, CDCl$_3$) 1.22 (1H, t, J 7.3 Hz, CH$_3$), 2.68-2.86 (2H, m, CH$_2$), 3.24 (1H, ddd, $J_{4,5}$ 9.7 Hz, $J_{5,6}$ 3.3 Hz, $J_{5,6'}$ 2.1 Hz, H-5), 3.56-3.60 (2H, m, H-6, H-6'), 3.61 (1H, at, J 9.1 Hz, H-3), 3.72 (1H, at, J 9.4 Hz, H-4), 3.89 (1H, at, J 9.1 Hz, H-2), 4.34 (1H, d, $J_{1,2}$ 9.7 Hz, H-1), 4.37, 4.31 (2H, Abq, J 12.2 Hz, PhCH$_2$), 4.56, 4.83 (2H, Abq, J 11.3 Hz, PhCH$_2$), 4.77-4.83 (2H, m, PhCH$_2$), 4.90 (1H, d, J 11.1 Hz, PhCHH), 4.97 (1H, d, J 10.7 Hz, PhCHH), 7.07-7.21 (14H, m, Ar-H), 7.25-7.27 (2H, m, Ar-H), 7.29-7.31 (2H, m, Ar-H), 7.36-7.38 (2H, m, Ar-H).

Example 13: *N*-Acetyl-L-cysteine (2,3,4,6-tetra-*O*-benzyl-1-dithio-β-D-glucopyranosyl disulfide)-L-serine methylester
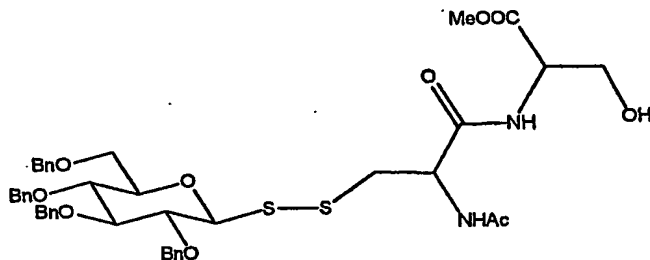
2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl phenylthiosulfonate (50 mg, 0.07 mmol) was dissolved in anhydrous DCM (5 mL) and stirred at RT under an atmosphere of Ar. To this *N*-acetyl-L-cysteine-L-serine methylester (19 mg, 0.07 mmol) and triethylamine (11 μL, 0.08 mmol) in anhydrous DCM (5 mL) and anhydrous methanol (0.5 mL) was slowly added dropwise *via* a syringe pump over a 5 h period. After a 5 h period, t.l.c. (ethyl acetate) indicated the formation of a major product (R_f 0.6) along with complete consumption of the starting material (R_f 0.9). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate) to afford the title product (48 mg, 82%) as a white crystalline solid; mp 96-97°C; $[\alpha]_D^{22}$ +56.2 (c, 1 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.03 (3H, s, $COCH_3$), 3.19 (1H, dd, $J_{CH,H}$ 14.0 Hz, $J_{CH,\alpha H}$ 8.3 Hz, CysC$\underline{H}$H), 3.37 (1H, dd, $J_{CH,H}$ 14.3 Hz, $J_{CH,\alpha H}$ 6.0 Hz, CysCH$\underline{H}$), 3.64 (1H, ddd, $J_{4,5}$ 9.6 Hz, $J_{5,6}$ 1.8 Hz, $J_{5,6'}$ 3.9 Hz, H-5), 3.72 (1H, at, J 9.2 Hz, H-4), 3.77 (1H, at, J 8.8 Hz, H-3), 3.82 (3H, s, OMe), 3.84-3.90 (4H, m, SerC$\underline{H}$H, H-2, H-6, H-6'), 3.96 (1H, dd, $J_{CH,H}$ 11.7 Hz, $J_{CH,\alpha H}$ 3.3 Hz, SerCH$\underline{H}$), 4.50 (1H, d, $J_{1,2}$ 9.6 Hz, H-1), 4.51, 4.70 (2H, ABq, J 11.6 Hz, PhC$\underline{H}_2$), 4.55, 4.85 (2H, ABq, J 10.4 Hz, PhC$\underline{H}_2$), 4.59-4.62 (1H, m, αHSer), 4.81, 4.87 (2H, ABq, J 10.6 Hz, PhC$\underline{H}_2$), 4.91, 4.97 (2H, ABq, J 11.0 Hz, PhC$\underline{H}_2$), 4.93-4.98 (1H, m, αHCys), 6.88 (1H, bd, $J_{NH,H}$ 7.9 Hz, NHAc), 7.13-7.39 (20H, m, 20 x Ar-C), 7.48 (1H, d, $J_{NH,H}$ 7.6 Hz, NHSer).
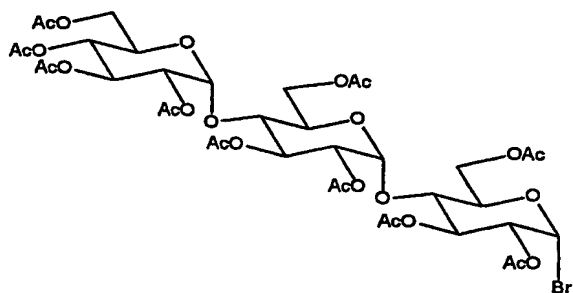
<u>Example 14: 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-D-glucopyranose</u>
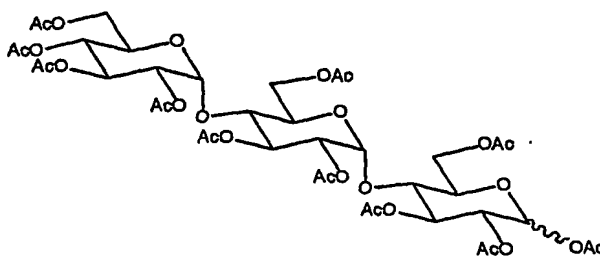
Sodium acetate (700 mg, 8.3 mmol) was added to acetic anhydride (50 mL) and heated to reflux, at which point maltotriose (3.00 g, 6.0 mmol) was added and stirred vigorously. After 90 min, t.l.c. (petrol:ethyl acetate, 1:2) indicated the formation of a product (R_f 0.3) with complete consumption of the starting material (R_f 0.0). The reaction was allowed to cool to RT and diluted with DCM (50 mL) and partitioned with water (100 mL). The phases were separated and the aqueous layer was re-extracted with DCM (2 x 50 mL). The combined organic layers were washed with sodium hydrogen carbonate (400 mL of a saturated aqueous solution) until pH 8 was obtained, brine (200 mL), dried (MgSO$_4$), filtered and concentrated *in vacuo* to afford the title product as a mixture of anomers (α/β, 2/11) as an amorphous white solid; for β compound δ_H (500 MHz, CDCl$_3$) 2.05, 2.07, 2.10, 2.14, 2.15, 2.19, 2.21, 2.27 (30H, 8 x s, 10 x OAc), 3.92 (1H, ddd, $J_{4,5}$ 9.5 Hz, $J_{5,6}$ 2.9 Hz, $J_{6,6}$ 4.1 Hz, H-5a), 3.95-4.01 (3H, m, H-4b, H-5b, H-5c), 4.05 (1H, at, J 9.1 Hz, H-4a), 4.09 (1H, dd, $J_{5,6}$ 2.5 Hz, $J_{6,6'}$ 12.7 Hz, H-6c), 4.21 (1H, dd, $J_{5,6}$ 3.4 Hz, $J_{6,6'}$ 12.6 Hz, H-6b), 4.29 (1H, dd, $J_{5,6}$ 3.4 Hz, $J_{6,6'}$ 12.4 Hz, H-6'c), 4.35 (1H, dd, $J_{5,6}$ 4.3 Hz, $J_{6,6'}$ 12.3 Hz, H-6a), 4.48-4.52 (2H, m, H-6'a, H-6'b), 4.78 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.3 Hz, H-2b), 4.90 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.6 Hz, H-2c), 5.01 (1H, dd, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.0 Hz, H-2a), 5.11 (1H, at, J 10.1 Hz, H-4c), 5.31 (1H, d, $J_{1,2}$ 3.9 Hz, H-1b), 5.32-5.44 (3H, m, H-3a, H-3b, H-3c), 5.45 (1H, d, $J_{1,2}$ 4.1 Hz, H-1c), 5.79 (1H, d, $J_{1,2}$ 8.2 Hz, H-1a); for α

compound selected data only, δ_H (500 MHz, CDCl$_3$) 2.08, 2.09, 2.12, 2.18, 2.21, 2.23, 2.26 (30H, 8 x s, 10 x OAc), 5.07 (1H, at, J 9.9 Hz), 6.28 (1H, d, $J_{1,2}$ 3.8 Hz, H-1a). Remaining signals lie in the following multiplet regions, 3.85-3.89, 3.90-3.98, 3.99-4.07, 4.15-4.18, 4.23-4.27, 4.29-4.32, 4.43-4.49, 4.74-4.76, 4.84-4.87, 4.98-4.94, 5.25-5.54; m/z (ES+) 984 (MNH$_4^+$, 30%), 989 (MNa$^+$, 100%); m/z HRMS (ES$^+$) Calcd. For $C_{40}H_{58}O_{27}N$ (MNH$_4^+$) 984.3196 Found 984.3199.
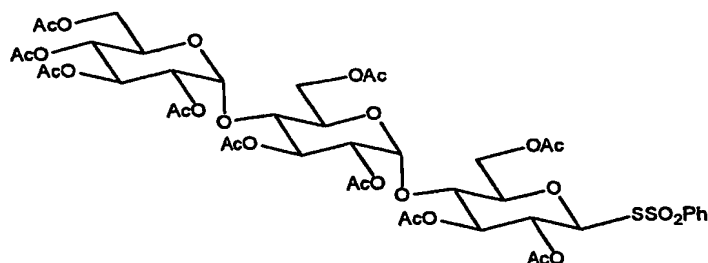
Example 15: 2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl bromide

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-D-glucopyranose (200 mg, 0.21 mmol) was dissolved in anhydrous DCM (5 mL). To this hydrogen bromide (33% in acetic acid, 2 mL) was added. The mixture was left under argon at RT. After a 30 min period, t.l.c. (petrol:ethyl acetate, 1:2) indicated the formation of a product (R$_f$ 0.6) with complete consumption of the starting material (R$_f$ 0.3). The reaction mixture was partitioned between DCM (10 mL) and water (10 mL), and the aqueous layer re-extracted with DCM (3 x 10 mL). The combined organic layers were washed with sodium hydrogen carbonate (20 mL of a saturated aqueous solution) until pH 8 was obtained, brine (20 mL), dried (MgSO$_4$), filtered and concentrated *in vacuo* to afford the title product (203 mg, 98%) as a white foam; [α]$_D^{22}$ +152.2

(c, 1.0 in CHCl$_3$); δ_H (400 MHz, CDCl$_3$) 2.03, 2.05, 2.06,
2.08, 2.10, 2.13, 2.18, 2.21 (30H, 10 x COCH$_3$), 3.93-3.99
(3H, m, H-4b, H-5a, H-5b), 4.05-4.10 (2H, m, H-4c,
H-6a), 4.20 (1H, dd, $J_{5,6}$ 1.8 Hz, $J_{6,6'}$ 12.2 Hz, H-6b),
4.26-4.34 (2H, m, H-5c, H-6a'), 4.35 (1H, dd, $J_{5,6}$ 3.5
Hz, $J_{6,6'}$ 12.7 Hz, H-6c), 4.52 (1H, dd, $J_{5,6}$ 0.6 Hz, $J_{6,6'}$
12.2 Hz, H-6b'), 4.57 (1H, dd, $J_{5,6}$ 2.1 Hz, $J_{6,6'}$ 12.4 Hz,
H-6c''), 4.74 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 9.9 Hz, H-2c),
4.78 (1H, dd, $J_{1,2}$ 4.2 Hz, $J_{2,3}$ 10.2 Hz, H-2b), 4.88 (1H,
dd, $J_{1,2}$ 4.0 Hz, $J_{2,3}$ 10.5 Hz, H-2a), 5.10 (1H, at, J
9.7 Hz, H-4a), 5.32 (1H, d, $J_{1,2}$ 4.0 Hz, H-1b), 5.39 (1H,
at, J 9.9 Hz, H-3q), 5.43-5.46 (1H, m, H-3b), 5,45 (1H,
d, $J_{1,2}$ 3.8 Hz, H-1a), 5.64 (1H, at, J 9.5 Hz, H-3c), 6.53
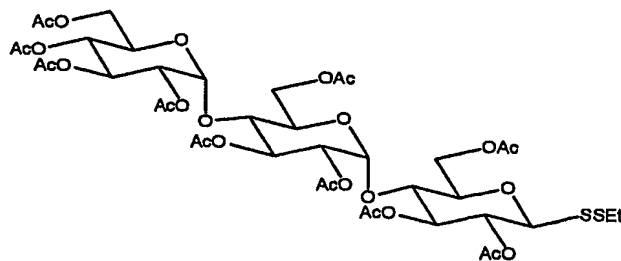(1H, d, $J_{1,2}$ 3.9 Hz, H-1c).

Example 16: 2,3,6-Tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-O-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranosyl phenylthiosulfonate

2,3,6-Tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,4,6-
tetra-O-acetyl-α-O-glucopyranosyl)-α-D-glucopyranosyl)-α-
D-glucopyranosyl bromide (200 mg, 0.21 mmol) was
dissolved in anhydrous acetonitrile (10 mL). To this
sodium benzenethiosulfonate (80 mg, 0.41 mmol) and
tetrabutylammonium iodide (10 mg, 0.02 mmol) were added.
The resulting mixture was stirred under argon at 70°C.
After a 2 h period, t.l.c. (petrol:ethyl acetate, 1:2)
indicated the formation of a UV active product (R$_f$ 0.5)

with complete consumption of the starting material (R_f 0.5). At which point the solution was allowed to cool to RT and filtered, the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 1:2) to afford the title product (140 mg, 62%) as a white amorphous solid; $[\alpha]_D^{22}$ +69.9 (c, 0.75 in $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 2.03, 2.04, 2.06, 2.08, 2.11, 2.15, 2.19, (30H, 10 x $COCH_3$), 3.77-3.79 (1H, m, H-5a), 3.94-4.00 (4H, m, H-4a, H-4c, H-5b, H-5c), 4.10 (1H, dd, $J_{5,6}$ 2.1 Hz, $J_{6,6'}$ 12.4 Hz, H-6b), 4.17-4.22 (3H, m, H-6a, H-6c, H-6a'), 4.29 (1H, dd, $J_{5,6}$ 3.3 Hz, $J_{6,6'}$ 12.6 Hz, H-6b'), 4.46 (1H, dd, $J_{5,6}$ 1.9 Hz, $J_{6,6'}$ 12.4 Hz, H-6c'), 4.76 (1H, dd, $J_{1,2}$ 3.9 Hz, $J_{2,3}$ 10.4 Hz, H-2a), 4.89-4.94 (2H, m, H-2b, H-2c), 5.12 (1H, at, J 9.9 Hz, H-4b), 5.28 (1H, d, $J_{1,2}$ 3.8 Hz, H-1a), 5.34 (1H, d, $J_{1,2}$ 9.7 Hz, H-1c), 5.37 (1H, at, J 9.1 Hz, H-3c), 5.41 (1H, at, J 10.1 Hz, H-3b), 5.41-5.45 (2H, m, H-1b, H-3a), 7.62-7.65 (2H, m, Ar-H), 7.71 (1H, m, Ar-H), 8.00-8.02 (2H, m, Ar-H).
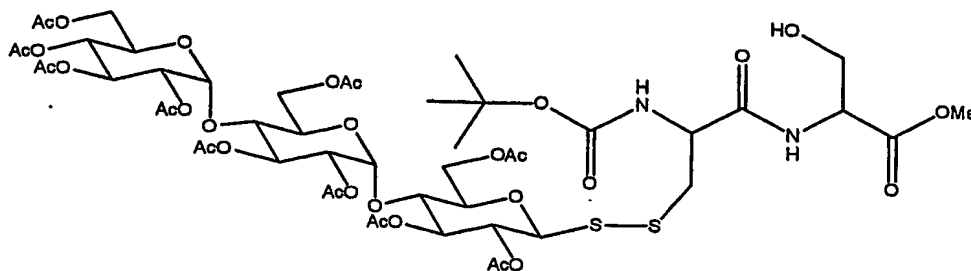
Example 17: Ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-1-dithio-β-D-glucopyranosyl disulfide

2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranosyl phenylthiosulfonate (50 mg, 0.05 mmol) was dissolved in anhydrous DCM (10 mL) and stirred at RT under an atmosphere of argon. A solution of

triethylamine (7 μL, 0.05 mmol) and ethane thiol (3 μL, 0.05 mmol) and anhydrous DCM (10 mL) was slowly added dropwise *via* a syringe pump over a 1 h period. After a 1 h period, t.l.c. (petrol:ethyl acetate, 1:2) indicated the formation of a major product (R_f 0.6) along with complete consumption of the starting material (R_f 0.4). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 1:2) to afford ethyl the title product (43 mg, 93 %) as a clear oil; $[\alpha]_D^{24}$ +26.4 (c, 1.5 in CHCl$_3$); δ_H (500 MHz, CDCl$_3$) 1.30 (1H, t, J 7.2 Hz, CH$_3$), 2.04, 2.05, 2.06, 2.07, 2.10, 2.14, 2.19, 2.20 (30H, 8 x s, 10 x COCH$_3$), 2.75-2.87 (2H, m, C$\underline{H}_2$CH$_3$), 3.77-3.81 (1H, m, H-5a), 3.96-4.00 (3H, m, H-4b, H-5c, H-5b), 4.03 (1H, at, J 9.3 Hz, H-4a), 4.10 (1H, dd, $J_{5,6}$ 2.3 Hz, $J_{6,6'}$ 12.6 Hz, H-6c), 4.22 (1H, dd, $J_{5,6}$ 2.9 Hz, $J_{6,6'}$ 12.4 Hz, H-6b), 4.29 (1H, dd, $J_{5,6}$ 3.7 Hz, $J_{6,6'}$ 12.4 Hz, H-6'c), 4.33 (1H, dd, $J_{5,6}$ 4.4 Hz, $J_{6,6}'$12.4 Hz, H-6a), 4.51 (1H, dd, $J_{5,6'}$ 1.8 Hz, $J_{6,6'}$ 12.4 Hz, H-6b', 4.57 (1H, dd, $J_{5,6}$ 2.3 Hz, $J_{6,6'}$ 12.4 Hz, H-6a'), 4.58 (1H, d, $J_{1,2}$ 9.9 Hz, H-1a), 4.79 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.6 Hz, H-2b), 4.90 (1H, dd, $J_{1,2}$ 4.3 Hz, $J_{2,3}$ 10.4 Hz, H-2c), 5.11 (1H, at, J 9.9 Hz, H-4c), 5.16 (1H, at, J 9.5 Hz, H-2a), 5.33 (1H, d, $J_{1,2}$ 4.1 Hz, H-1b), 5.37 (1H, at, J 8.9 Hz, H-3a), 5.38-5.44 (2H, m, H-3b, H-3c), 5.45 (1H, d, $J_{1,2}$ 4.1 Hz, H-1c).

Example 18: *N*-Butoxycarbonyl-L-cysteine (2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-1-dithio-β-D-glucopyranosyl disulfide)-L-serine methylester

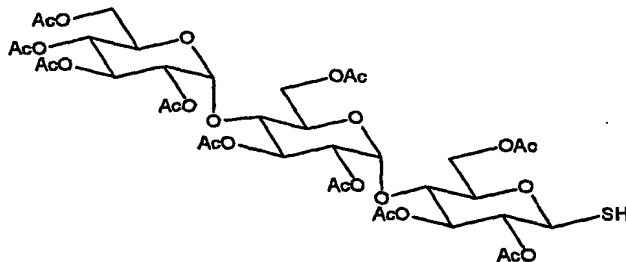2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranosyl phenylthiosulfonate (89 mg, 0.08 mmol) was dissolved in anhydrous DCM (5 mL) and stirred at RT under an atmosphere of argon. A solution of triethylamine (0.014 mL, 0.2 mmol) and *N*-butoxycarbonyl-L-cysteinyl-L-serine methylester (30 mg, 0.09 mmol) in anhydrous DCM (10 mL) and anhydrous methanol (1 mL) was slowly added dropwise *via* a syringe pump over a 3 h period. After a 3 h period, t.l.c. (ethyl acetate) indicated the formation of a major product (R_f 0.6) along with complete consumption of the starting material (R_f 0.7). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate) to afford the title product (66 mg, 74%) as an amorphous white solid; $[\alpha]_D^{24}$ +25.1 (c, 1.25 in CHCl$_3$); δ_H (500 MHz, CDCl$_3$) 1.47 (9H, s, C(CH$_3$)$_3$), 2.00, 2.01, 2.02, 2.03, 2.06, 2.09, 2.15, 2.18 (30H, 8 x s, 10 x COCH$_3$), 2.75-2.87 (1H, m, C$\underline{H}$HCys), 3.16-3.19 (1H, m, CH$\underline{H}$Cys), 3.27 (1H, t, *J* 6.2 Hz, OH), 3.81 (3H, s, OMe), 3.83-3.85 (1H, m, H-5a), 3.92-4.01 (6H, m, H-4b, H-5b, H-5c, H6a, H-6a', CH$\underline{H}$Ser), 4.06 (1H, dd, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.2 Hz, H-6c), 4.09-4.16 (2H, m, H-4a, H-6b), 4.25 (1H, dd, $J_{5,6}$ 3.2 Hz, $J_{6,6'}$ 12.3 Hz, H-6c'), 4.39-4.41 (1H, m, CH$\underline{H}$Ser), 4.52-4.67 (4H, m, αHSer, αHCys, H-1a, H-6'b), 4.74 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.3 Hz, H-2b), 4.85 (1H, dd, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 10.5 Hz, H-2c), 5.07 (1H, at, *J* 9.9 HZ, H-4c), 5.11-5.13 (1H, m, H-2a), 5.28 (1H, d, $J_{1,2}$ 4.1 Hz, H-1b), 5.32-5.41 (4H, m, H-3a, H-3b, H-3c,

NHCys), 5.42 (1H, d, $J_{1,2}$ 3.9 Hz, H-1c), 7.25 (1H, bd, $J_{NH,\alpha H}$ 6.7 Hz, NHSer).

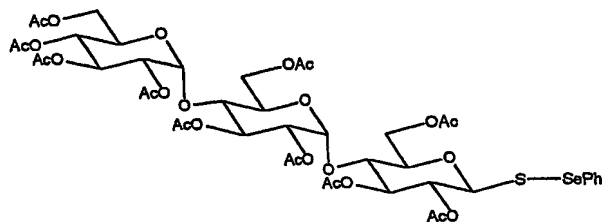Example 19: 2,3,6-Tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-O-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranosylthiol

2,3,6-Tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-O-glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl bromide (2.10 g, 2.10 mmol) was dissolved in anhydrous acetone (60 mL). To this anhydrous thiourea (315 mg, 4.2 mmol) was added and then heated to reflux under an atmosphere of argon. After a 6.5 h period, t.l.c. (petrol:ethyl acetate, 1:2) indicated the formation of a product (R_f 0.0) with complete consumption of the starting material (R_f 0.3). The reaction was concentrated in vacuo and titurated with DCM to remove the organics from the excess thiourea. The filtrate was concentrated in vacuo and the residue was purified by column flash chromatography (ethyl acetate/methanol, 9:1) to afford the intermediate 2,3,6-tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-O-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranosyl-1-sothiouronium bromide (1.14g, 50%) which was carried on without characterisation. 2,3,6-tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-O-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranosyl-1-sothiouronium bromide (100 mg, 0.09 mmol) and $Na_2S_2O_5$ (22 mg, 0.11 mmol) were added to a

stirred mixture of DCM (30 mL) and water (15 mL). The mixture was heated to reflux under argon. After 2.5 h, t.l.c. (petrol:ethyl acetate, 1:2) indicated the formation of a product (R_f 0.4) with complete consumption of the starting material (R_f 0.0), at which point the reaction was cooled to RT and the phases separated. The aqueous layer was re-extracted with DCM (2 x 20 mL). The combined organic layers were washed with brine (20 mL), dried (MgSO$_4$), filtered and the solvent removed *in vacuo*. To afford the title product (74 mg, 84%) as a white amorphous solid; $[\alpha]_D^{22}$ +99.5 (c, 1.0 in CHCl$_3$); δ_H (400 MHz, CDCl$_3$) 1.99, 2.00, 2.01, 2.02, 2.03, 2.05, 2.10, 2.15, 2.18 (30H, 9 x s, 10 x COCH$_3$), 3.72-3.76 (1H, m, H-5a), 3.90-4.00 (4H, m, H-4a, H-4b, H-5b, H-5c), 4.05 (1H, dd, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.3 Hz, H-6c), 4.17 (1H, dd, $J_{5,6}$ 3.3 Hz, $J_{6,6'}$ 12.3 Hz, H-6b), 4.25 (1H, dd, $J_{5,6}$ 3.6 Hz, $J_{6,6'}$ 12.5 Hz, H-6c'), 4.30 (1H, $J_{5,6}$ 4.3 Hz, $J_{6,6'}$ 12.2 Hz, H-6c), 4.44 (1H, dd, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.1 Hz, H-6a'), 4.46 (1H, dd, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.2 Hz, H-6b'), 4.59 (1H, d, $J_{1,2}$ 9.7 Hz, H-1a), 4.74 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.6 Hz, H-2b), 4.80 (1H, at, J 9.0 Hz, H-2a), 4.85 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.6 Hz, H-2c), 5.07 (1H, at, J 9.9 Hz, H-4c), 5.25 (1H, at, J 9.0 Hz, H-3a), 5.26 (1H, d, $J_{1,2}$ 4.1 Hz, H-1b), 5.35 (1H, at, J 10.0 Hz, H-3b), 5.37-5.41 (2H, m, H-1c, H-3c).

Example 20: Phenyl 2,3,6-tri-O-acetyl-1-selenenylsulfide-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-O-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranoside

2,3,6-Tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,4,6-
tetra-O-acetyl-α-O-glucopyranosyl)-α-D-glucopyranosyl)-β-
D-glucopyranosylthiol (500 mg, 0.53 mmol) and phenyl
selenium bromide (200 mg, 0.9 mmol) were dissolved in
anhydrous DCM (20 ml). After a 5 min period, t.l.c.
(petrol:ethyl acetate 1:2) indicated the formation of a
major product (R_f 0.4) along with complete consumption of
the starting material (R_f 0.3). The reaction was quenched
with the addition of triethylamine (5 ml) and then
concentrated *in vacuo*. The residue was purified by flash
column chromatography (petrol:ethyl acetate 1:2) to
afford the title product (527 mg, 91%) as an amorphous
off white solid; $[\alpha]_D^{25}$ -2.6 (c, 1.0 in $CHCl_3$); δ_H (400
MHz, $CDCl_3$), 1.99, 2.01, 2.02, 2.04, 2.06, 2.10, 2.14
(30H, 9 x s, 10 x OAc), 3.79 (1H, dat, $J_{4,5}$ 9.7 Hz, J
3.4 Hz, H-5a), 3.92 (3H, m, H4b, H-5b, H-5c), 4.00 (1H,
at, J 9.3 Hz, H-4a), 4.05 (1H, dd, $J_{5,6}$ 2.8 Hz, $J_{6,6'}$
12.8 Hz, H-6c), 4.15 (1H, dd, $J_{5,6}$ 2.8 Hz, $J_{6,6'}$ 12.6 Hz,
H-6b), 4.22 (1H, dd, $J_{5,6}$ 3.7 Hz, $J_{6,6'}$ 12.0 Hz, H-6a), 4.25
(1H, dd, $J_{5,6}$ 3.3 Hz, $J_{6,6'}$ 12.0 Hz, H-6c'), 4.42-4.46 (2H,
m, H-6a', H-6b'), 4.66 (1H, d, $J_{1,2}$ 9.9 Hz, H-1a), 4.74
(1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.4 Hz, H-2b), 4.86 (1H, dd,
$J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.5 Hz, H-2c), 5.06 (1H, at, J 9.6 Hz,
H-4c), 5.07 (1H, at, J 9.8 Hz, H-2a), 5.27 (1H, d, $J_{1,2}$
4.4 Hz, H-1b), 5.32-5.39 (3H, m, H-3a, H-3b, H-3c), 5.41
(1H, d, $J_{1,2}$ 4.2 Hz, H-1c), 7.27-7.29 (3H, m, Ar-H),
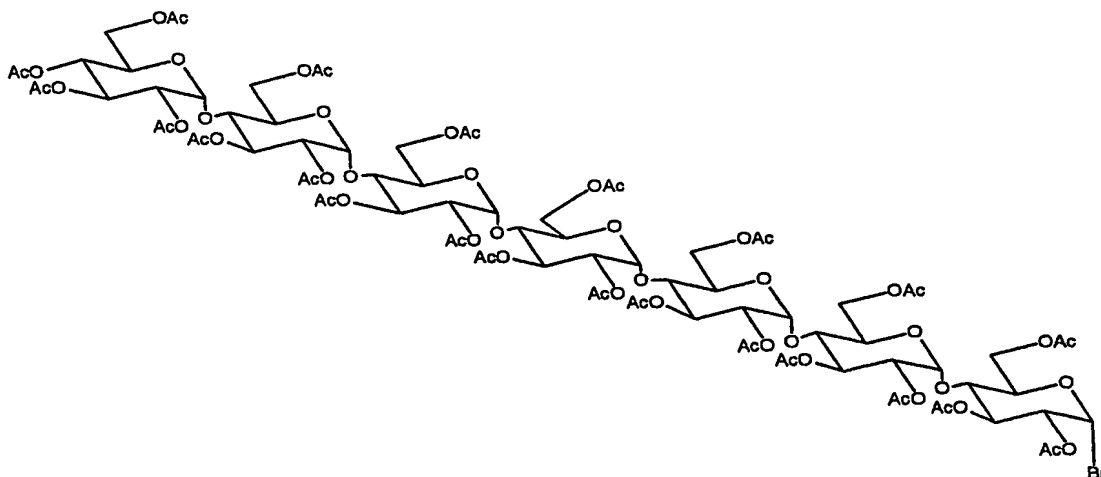7.64-7.67 (2H, m, Ar-H).

Example 21: 2,3,6-Tetra-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-O-glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl)-D-glucopyranose
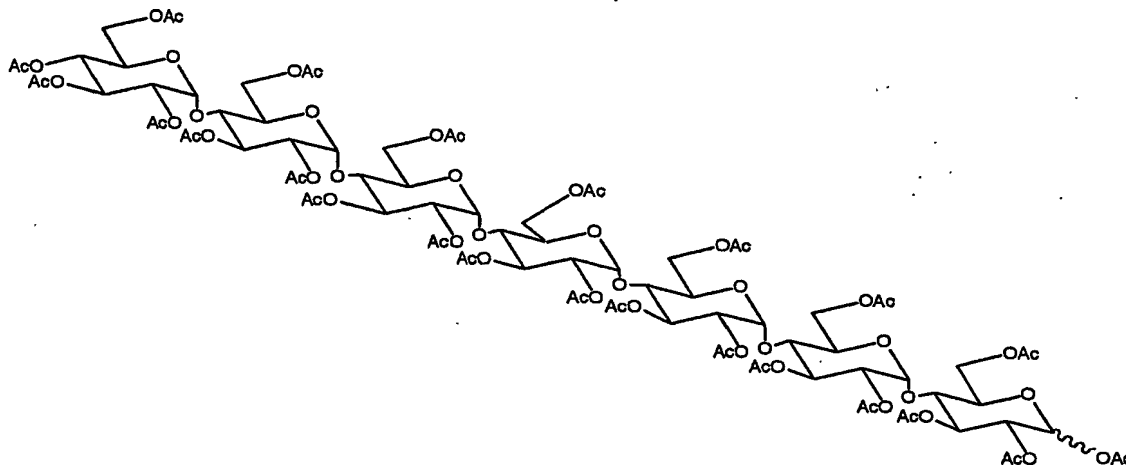
Sodium acetate (420 mg, 5.2 mmol) was added to acetic anhydride (30 mL) and heated to reflux. At which point maltoheptose (1.00 g, 0.86 mmol) was added and stirred vigorously. After 90 min t.l.c. (petrol:ethyl acetate, 1:3) indicated the formation of a product (R_f 0.3) with complete consumption of the starting material (R_f 0.0). The reaction was allowed to cool to RT, diluted with DCM (50 mL) and partitioned with water (100 mL). The phases were separated and the aqueous layer was re-extracted with DCM (2 x 40 mL). The combined organic layers were washed with sodium hydrogen carbonate (200 mL of a saturated aqueous solution) until pH 8 was obtained, brine (100 mL), dried (MgSO$_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (petrol:ethyl acetate, 1:3) to afford the title product as a mixture of anomers as an amorphous white solid (α/β, 0.15/0.85); δ_H (500 MHz, CDCl$_3$) 2.02, 2.03, 2.04, 2.05, 2.06, 2.07, 2.08, 2.10, 2.13, 2.19, 2.22, 2.24 (66H, 12 x s, 22 x OAc), 3.89-4.14 (13H, m,

H-4a, H-4b, H-4c, H-4d, H-4e, H-4f, H-5a, H-5b, H-5c,
H-5d, H-5e, H-5f, H-5g), 4.25-4.34, 4.39 (1H, dd,
J 4.0 Hz, J 12.3 Hz), 4.52-4.56 (13H, m, H-6a, H-6a',
H-6b, H-6b', H-6c, H-6c', H-6d, H-6d', H-6e, H-6e',
H-6f, H-6f', H-6d, H-6g'), 4.75-4.79 (5H, m, H-2b, H-2c,
H-2d, H-2e, H-2e, H-2f), 4.90 (1H, dd, $J_{1,2}$ 3.7 Hz, $J_{2,3}$
10.5 Hz, H-2g), 5.00 (1H, at, J 9.4 Hz, H-4g), 5.31-5.45
(13H, m, H-3a, H-3b, H-3c, H-3d, H-3e, H-3f, H-3g, H-1b,
H-1c, H-1d, H-1e, H-1f, H-1g), 5.79 (0.85H, d, $J_{1,2}$
8.1 Hz, H-1aβ), 6.28 (0.15H, d, $J_{1,2}$ 4.0 Hz, H-1aα).

Example 22: 2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-
4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-
(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-
(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-
glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl)-
α-D-glucopyranosyl)- α-D-glucopyranosyl)-α-D-
glucopyranosyl bromide

2,3,6-Tetra-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-
tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-
acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-
acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-α-D-
glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl)-

α-D-glucopyranosyl)-D-glucopyranose (100 mg, 0.05 mmol) was dissolved in anhydrous DCM (5 mL). To this hydrogen bromide (33% in acetic acid, 0.5 mL) was added. The mixture was left stirring under an atmosphere argon at RT. After a 40 min period, t.l.c. (petrol:ethyl acetate, 1:3) indicated the formation of a product (R_f 0.7) with complete consumption of the starting material (R_f 0.3). The reaction mixture was partitioned between DCM (10 mL) and water (10 mL), and the aqueous layer re-extracted with DCM (3 x 10 mL). The combined organic layers were washed with sodium hydrogen carbonate (150 mL of a saturated aqueous solution) until pH 7 was obtained, brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford the title product (98 mg, 96%) as a white foam; $[\alpha]_D^{22}$ +162.0 (c, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.02, 2.03, 2.04, 2.06, 2.08, 2.10, 2.11, 2.14, 2.19, 2.23, 2.24, 2.25 (66H, 12 x s, 22 x OAc), 3.94-4.04 (12H, m, H-4b, H-4c, H-4d, H-4e, H-4f, H-5b, H-5c, H-5d, H-5e, H-5f, H-5g), 4.08 (1H, dd, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.6 Hz, H-6), 4.19-4.33, 4.53-4.60 (12H, m, H-5a, H-6b, H-6b', H-6c, H-6c', H-6d, H-6d', H-6e, H-6e', H-6f, H-6f', H-6g, H-6g'), 4.12 (1H, at, J 9.5 Hz, H-4a), 4.40 (1H, dd, $J_{5,6}$ 3.1 Hz, $J_{6,6'}$ 12.7 Hz, H-6a), 4.64 (1H, dd, $J_{5,6}$ 2.3 Hz, $J_{6,6'}$ 12.5 Hz, H-6a'), 4.74 (1H, dd, $J_{1,2}$ 3.9 Hz, $J_{2,3}$ 9.7 Hz, H-2a), 4.75-4.97 (5H, m, H-2b, H-2c, H-2d, H-2e, H-2f), 4.89 (1H, d, $J_{1,2}$ 4.0 Hz, $J_{2,3}$ 10.6 Hz, H-2g), 5.11 (1H, at, J 9.9 Hz, H-4g), 5.32-5.47 (12H, m, H-1b, H-1c, H-1d, H-1e, H-1f, H-1g, H-3b, H-3c, H-3d, H-3e, H-3f, H-3g), 5.65 (1H, at, J 9.4 Hz, H-3a), 6.54 (1H, d, $J_{1,2}$ 4.3 Hz, H-1a).

Example 23: 2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl)-

α-D-glucopyranosyl)- α-D-glucopyranosyl)-β-D-
glucopyranosylthiol
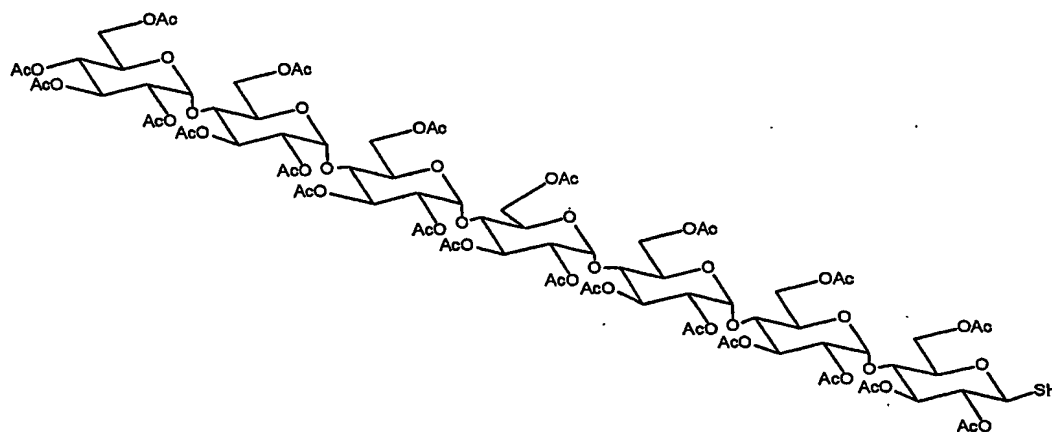
2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-
tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-
acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-
acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-α-D-
glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl)-
α-D-glucopyranosyl)-α-D-glucopyranosyl bromide (1.08 g,
0.5 mmol) and tetrabutylammonium iodide (19 mg,
0.05 mmol) was dissolved in anhydrous acetone (50 mL).
To this dried thiourea (52 mg, 0.7 mmol) was added and
then heated to reflux under an atmosphere of argon.
After a 8 h period, t.l.c. (petrol:ethyl acetate, 1:4)
indicated the formation of a minor product (R_f 0.0)
with complete consumption of the starting material (R_f
0.6). The reaction was concentrated *in vacuo* and
titurated with DCM to remove the organics from the
excess thiourea. The filtrate was concentrated *in vacuo*
and the residue was purified by column flash
chromatography (ethyl acetate/methanol, 9:1) to afford
the intermediate 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-
acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-
4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-
(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-

glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl)-
α-D-glucopyranosyl)-α-D-glucopyranosyl)-β-D-
glucopyranosyl-1-isothiouronium bromide (212 mg, 19%)
which was taken on further without characterisation.
2,3,6-Tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,6-
tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,6-tri-O-
acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-
acetyl-α-O-glucopyranosyl)-α-D-glucopyranosyl)-α-D-
glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl)-
α-D-glucopyranosyl)-β-D-glucopyranosyl-1-isothiouronium
bromide (210 mg, 0.09 mmol) and $Na_2S_2O_5$ (22 mg,
0.11 mmol) were added to a stirred mixture of DCM
(10 mL) and water (5 mL). The mixture was heated to
reflux under argon. After 4.5 h, t.l.c. (petrol:ethyl
acetate, 1:2) indicated the formation of a product (R_f
0.2) with complete consumption of the starting material
(R_f 0.0), at which point the reaction was cooled to RT
and the phases separated. The aqueous layer was
re-extracted with DCM (2 x 10 mL). The combined organic
layers were washed with brine (20 mL), dried ($MgSO_4$),
filtered and the solvent removed in $vacuo$ to afford the
title product (185 mg, 90%) as a white amorphous solid;
$[\alpha]_D^{24}$ +128.1 (c, 1.0 in $CHCl_3$); δ_H (500 MHz, $CDCl_3$), 2.00,
2.01, 2.02, 2.03, 2.04, 2.05, 2.07, 2.08, 2.12, 2.17,
2.19, 2.21, 2.22, 2.23 (66H, 14 x s, 22 x $COCH_3$), 2.27
(1H, d, $J_{1,SH}$ 9.8 Hz, SH), 3.76 (1H, dat, $J_{4,5}$ 9.7 Hz, J
3.5 Hz, H-5a), 3.92-4.08 (12H, m, H-4a, H-4b, H-4c,
H-4d, H-4e, H-4f, H-5b, H-5c, H-5d, H-5e, H-5f, H-5g),
4.17-4.36, 4.49-4.56 (12H, m, H-6b, H-6b', H-6c, H-6c',
H-6d, H-6d', H-6e, H-6e', H-6f, H-6f', H-6g, H-6g'),
4.39 (1H, dd, $J_{5,6}$ 3.6 Hz, $J_{6,6'}$ 12.2 Hz, H-6a), 4.48 (1H,
dd, $J_{5,6}$ 3.2 Hz, $J_{6,6'}$ 12.3 Hz, H-6a), 4.62 (1H, at, J
9.5 Hz, H-1a), 4.73-4.78 (5H, m, H-2b, H-2c, H-2d, H-2e,
H-2f), 4.82 (1H, at, J 9.5 Hz, H-2a), 4.88 (1H, dd, $J_{1,2}$
4.0 Hz, $J_{2,3}$ 10.4 Hz, H-2g), 5.09 (1H, at, J 9.9 Hz,
H-4g), 5.27 (1H, at, J 9.1 Hz, H-3a), 5.30-5.44 (12H, m,

-1b, H-1c, H-1d, H-1e, H-1f, H-1g, H-3b, H-3c, H-3d, H-3e, H-3f, H-3g).

## Example 24: bis-N-Butoxycarbonyl-L-cysteinyl-L-threonine methylester
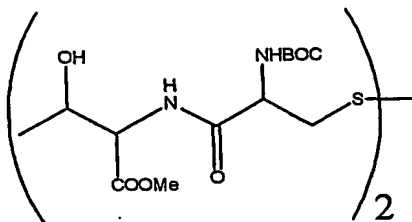
bis-N-Butoxycarnoyl-L-Cysteine (4.0 g, 9.1 mmol), L-threonine methylester (2.42 g, 18.2 mmol), DCC (3.75 g, 18.2 mmol) ,HOBt (2.46 g, 18.2 mmol) and DIPEA (2.5 ml, 18.2 mmol) was dissolved in freshly distilled DCM (150 mL). After a 18 h period, t.l.c. (ethyl acetate:methanol 9:1) indicated the formation of a major product (R_f 0.5) along with complete consumption of the starting material (R_f 0.0). The reaction was diluted with water (2 x 100 ml) and the phases were partitioned. The organics were washed with brine (100 ml), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate:methanol 9:1), and recrystallisation from methanol/diethyl ether afforded the title product (3.26 g, 60%) as a white crystalline solid; mp 145-147°C; $[\alpha]_D^{25}$ +20.8 (c, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$), 1.23 (3H, d, $J_{CH,CH3}$ 6.6 Hz, CHCH$_3$), 1.44 (9H, s, C(CH$_3$)$_3$), 3.11-3.12 (2H, m, CH$_2$Cys), 3.26 (1H, bs, OH), 3.75 (3H, s, OMe), 4.32-4.36 (1H, m, CHCH$_3$), 4.61 (dd, $J_{NH,\alpha Thr}$ 8.7 Hz, $J_{\alpha H,CHCH3}$ 2.15 Hz, CHCH$_3$), 4.63-4.68 (1H, m, αCys), 5.75 (1H, d, $J_{NH,\alpha HCys}$ 7.4 Hz, NHCys), 7.56 (1H, d, $J_{NH,\alpha Thr}$ 8.6 Hz, NHThr).

Example 25: *N*-Butoxycarbonyl-L-cysteinyl-L-threonine methylester
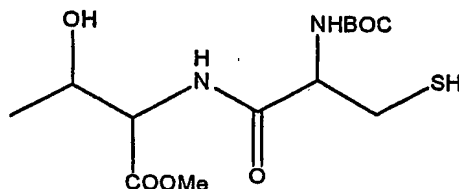

bis-*N*-Butoxycarbonyl-L-cysteinyl-L-threonine methylester (2.0 g, 3.3 mmol) was dissolved in wet chloroform (100 mL) and methanol (10 mL) and stirred. To this stirred solution tributylphosphine (1.0 mL, 4.0 mmol) was added. After a 2 h period, t.l.c. (ethyl acetate:methanol 9:1) indicated the formation of a product (R_f 0.8) along with complete consumption of the starting material (R_f 0.7). The reaction was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate) to afford the title product (2.0 g, 99%) as a white foam; $[\alpha]_D^{25}$ -11.4 (c, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.09 (3H, d, $J_{CH,CH3}$ 6.4 Hz, CH_3), 1.34 (9H, s, $C(CH_3)_3$), 1.65 (1H, at, J 8.7 Hz, SH), 2.72-2.89 (2H, m, CH_2), 3.66 (3H, s, OMe), 3.96 (1H, m, OH), 4.24-4.28 (1H, m, C$\underline{H}$CH$_3$), 4.34-4.36 (1H, m, αHCys), 4.49 (1H, dd, $J_{\alpha HThr,NH}$ 8.5 Hz, $J_{\alpha HThr,CHCH3}$ 2.7 Hz, αHThr), 5.82 (1H, d, $J_{\alpha HCys,NH}$ 8.2 Hz, NHCys), 7.38 (1H, d, $J_{\alpha HThr,NH}$ 8.5 Hz, NHThr).

Example 26: *N*-butoxycarbonyl-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyranosyl disulfide)-L-threonine methylester
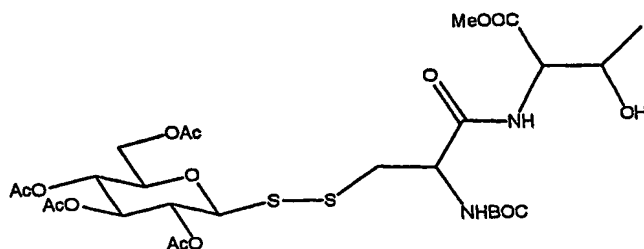
Phenyl 2,3,4,6-tetra-O-acetyl-1-selenenylsulfide-D-β-glucopyranoside (130 mg, 0.25 mmol) and triethylamine (0.02 mL, 0.18 mmol) were dissolved in freshly distilled DCM (10 mL). The resulting solution was stirred at RT. A solution of N-butoxycarbonyl-L-cysteine-L-threonine methylester (30 mg, 0.089 mmol) in anhydrous methanol (4 mL) was added slowly to the above solution. After a 10 min period, t.l.c. (petrol:ethyl acetate, 1:2) indicated the formation of a product (R_f 0.2) along with complete consumption of the starting material (R_f 0.5). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 1:2) to afford the title product (32 mg, 51%) as a white amorphous solid; $[\alpha]_D^{25}$ -81.2 (c, 0.25 in CHCl$_3$); δ_H (400 MHz, CDCl$_3$) 1.28 (3H, d, J_{CHCH3} 6.7 Hz, CHC$\underline{H}_3$), 1.51 (9H, s, C(CH$_3$)$_3$), 2.06, 2.08, 2.10 2.14 (12H, 4 x s, 4 x OAc), 2.86 (1H, bs, OH), 3.06 (1H, dd, $J_{CH\alpha H}$ 8.8 Hz, J_{CHCH} 13.4 Hz, C$\underline{H}$HCys), 3.31 (1H, dd, $J_{CH\alpha H}$ 4.2 Hz, J_{CHCH} 13.1 Hz, CH$\underline{H}$Cys), 3.82 (3H, s, OC$\underline{H}_3$), 3.87-3.89 (1H, m, H-5), 4.32-4.38 (2H, m, H-6, H-6'), 4.39 (1H, dd, J_{CHCH3} 6.4 Hz, $J_{CH\alpha H}$ 2.5 Hz, C$\underline{H}$OH), 4.60-4.65 (3H, m, H-1, αHThr, αHCys), 5.20-5.32 (3H, m, H-2, H-3, H-4), 5.42 (1H, d, $J_{NH\alpha H}$ 8.0 Hz, NHCys), 7.12 (1H, d, $J_{NH\alpha H}$ 8.9 Hz, NHThr).

Example 27: N-butoxycarbonyl-L-cysteine (2,3,4,6-tetra-O-acetyl-1-dithio-β-D-galactopyranosyl disulfide)-L-threonine methylester
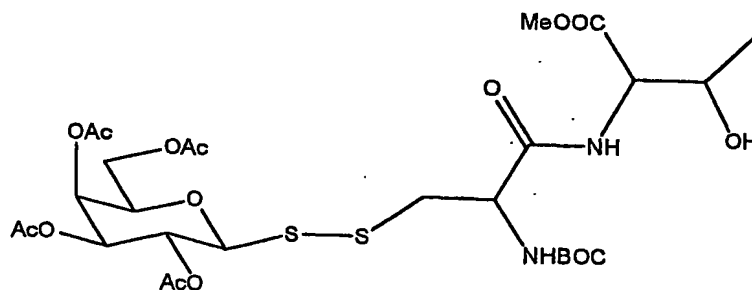
Phenyl 2,3,4,6-tetra-O-acetyl-1-selenenylsulfide-D-β-galactopyranoside (140 mg, 0.27 mmol) and triethylamine (0.01 mL, 0.089 mmol) were dissolved in freshly distilled DCM (5 mL). The resulting solution was stirred at RT. A solution of N-butoxycarbonyl-L-cysteine-L-threonine methylester (26 mg, 0.077 mmol) in anhydrous DCM (5 mL) and anhydrous methanol (4 mL) was added slowly to the above solution. After a 10 min period, t.l.c. (petrol:ethyl acetate, 1:2) indicated the formation of a product (R_f 0.2) along with complete consumption of the starting material (R_f 0.6). The solution was concentrated in $vacuo$. The residue was purified by flash column chromatography (petrol:ethyl acetate, 1:2) to afford the title product (49 mg, 93%) as a white amorphous solid; $[\alpha]_D^{25}$ -81.2 (c, 0.25 in CHCl$_3$); δ_H (400 MHz, CDCl$_3$) 1.24 (3H, d, $J_{CH,CH3}$ 6.4 Hz, CH$_3$), 1.46 (9H, s, C(CH$_3$)$_3$), 2.01, 2.06, 2.08, 2.20 (12H, 4 x s, 4 x OAc), 2.79 (1H, bd, $J_{CH,OH}$ 4.1 Hz, OH), 2.99 (1H, dd, $J_{\alpha H,CH2}$ 8.8 Hz, $J_{CH,H}$ 13.9 Hz, C$\underline{H}$HCys), 3.32-3.35 (1H, m, CH$\underline{H}$Cys), 3.76 (3H, s, OCH$_3$), 4.04 (1H, at, J 6.2 Hz, H-5), 4.10-4.16 (1H, m, H-6), 4.19 (1H, dd, $J_{5,6'}$ 6.1 Hz, $J_{6,6'}$ 10.8 Hz, H-6'), 4.36-4.46 (1H, m, C$\underline{H}$OH), 4.56 (1H, dd, $J_{\alpha HThr,CH}$ 2.4 Hz, $J_{\alpha H,NH}$ 8.9 Hz, αHThr), 4.57-4.64 (1H, m, αHCys), 4.65 (1H, d, $J_{1,2}$ 9.0 Hz, H-1), 5.13 (1H, dd, $J_{2,3}$ 9.8 Hz, $J_{2,3}$ 9.8 Hz, H-3), 5.31 (1H, d, $J_{\alpha HCys,NH}$ 8.3 Hz, NHCys), 5.47 (1H, d, $J_{3,4}$ 3.2 Hz, H-4), 5.52 (1H, at, J 9.6 Hz, H-2), 6.91 (1H, d, $J_{\alpha HThr,NH}$ 9.0 Hz, NHThr).

Example 28: Phenyl-1-selenenylsulfide-β-D-glucopyranoside
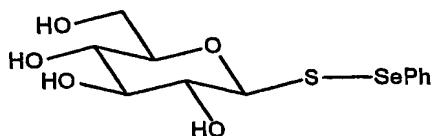
1-Thio-β-D-glucopyranoside (200 mg, 0.9 mmol) and phenylselenenyl bromide (230 mg, 1.0 mmol) were added to anhydrous 1,4-dioxane (5 mL) stirred under an atmosphere of argon. After a 1 min period, t.l.c. (ethyl acetate) indicated the formation of a major product (R_f 0.2). The reaction was quenched with the addition of triethylamine (2 mL). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate:methanol, 9:1) to afford the title product (165 mg, 57%) as an off white amorphous solid; $[\alpha]_D^{22}$ +56.2 (c, 1 in $CHCl_3$); δ_H (400 MHz, MeOD) 3.31-3.33 (2H, m, H-3, H-5), 3.39-3.45 (2H, m, H-2, H-4), 3.62 (1H, dd, $J_{5,6}$ 5.3 Hz, $J_{6,6'}$ 12.1 Hz, H-6), 3.83 (1H, dd, $J_{5,6'}$ 1.9 Hz, $J_{6,6'}$ 12.2 Hz, H-6), 4.47 (1H, d, $J_{1,2}$ 9.4 Hz, H-1), 7.27-7.34 (3H, m, Ar-H), 7.75-7.78 (2H, m, Ar-H).
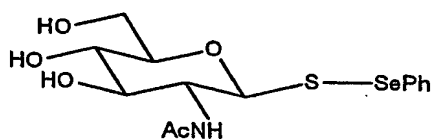
Example 29: 1-Thio-2-acetylamino-2-deoxy-β-D-glucopyranoside
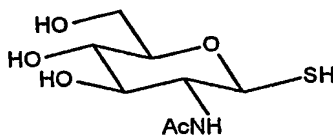
3,4,6-Tri-O-acetyl-2-acetylamino-2-deoxy-β-D-glucopyranosyl thiol (400 mg, 0.98 mmol) and sodium methoxide (18 mg, 0.03 mmol) were added to a stirred solution of methanol (5ml). After a 30 min period, t.l.c. (ethyl acetate) indicated the formation of a product (R_f 0.0) with complete consumption of the

starting material (R_f 0.2). The reaction was neutralised with the addition of dowex-50 ion exchange resin® after which point the reaction was filtered and concentrated *in vacuo*. Recrystallisation from methanol/ethyl acetate afforded the title product (13.35 g, 95%) as a white crystalline solid; m.p. 85-88°C [Lit. 86-88°C][18]; $[\alpha]_D^{22}$ – 10.4 (c, 1.0 in MeOH) [Lit. $[\alpha]_D^{25}$ +177.1 (c, 1.45 in CHCl$_3$)][18]; δ_H (400 MHz, MeOH), 2.00 (3H, s, CH$_3$), 3.27-3.37 (2H, m, H-4, H-5), 3.42 (1H, at J 9.1 Hz, H-3), 3.64-3.73 (2H, m, H2, H-6), 3.87 (1H, dd, $J_{5,6}$ 2.1 Hz, $J_{6,6'}$ 12.0 Hz, H-6'), 4.56 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 8.11 (1H, bd, $J_{NH,2}$ 9.1 Hz, NH).

Example 30: Phenyl-2-acetylamino-2-deoxy-1-selenenylsulfide-β-D-glucopyranoside

1-Thio-2-acetylamino-2-deoxy-β-D-glucopyranoside (230 mg, 0.98 mmol) and phenylselenenyl bromide (250 mg, 1.08 mmol) were added to anhydrous 1,4-dioxane (5 mL) and anhydrous methanol (3 ml) stirred under an atmosphere of argon. After a 1 min period, t.l.c. (ethyl acetate:methanol, 9:1) indicated the formation of a major product (R_f 0.4). The reaction was quenched with the addition of triethylamine (5 mL). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate:methanol, 9:1) to afford the title product (270 mg, 70%) as a white amorphous solid; $[\alpha]_D^{22}$ -174.0 (c, 1 in MeOH); δ_H (400 MHz, MeOD), 1.96 (3H, s, CH$_3$), 3.31-3.39 (2H, m, H-4, H-5), 3.51 (1H, at, J 8.1 Hz, H-3), 3.65 (1H, dd, $J_{5,6}$ 5.0 Hz, $J_{6,6'}$ 11.7 Hz, H-6), 3.82-3.90 (2H, m, H-2, H-6'), 4.65 (1H, d, $J_{1,2}$ 10.2 Hz, H-1), 7.27-7.34 (3H, m, ArH), 7.72-7.74 (2H, m, ArH).

Example 31: Protein glycosylation procedures using thiosulfonate reagents

A.   SBLS156C mutant (24 mg, 0.89 µmol) was dissolved in aqueous buffer solution (2.4 mL, 70 mM HEPES, 2 mM $CaCl_2$, pH 6.9). 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl phenylthiosulfonate (50mg, 0.1 mmol) was dissolved in water/acetonitrile (1.6 mL, 9/7 v/v). A portion of the sugar solution (50 µL) was added to the protein solution and placed on an end-over-end rotator. After 25 min, the absence of free thiol was shown by Ellman's analysis (Ellman, G. L. Arch. Biochem. Biophys. 1959, 82, 70), at which point another portion of sugar solution (50 µL) was added. The reaction was placed on an end-over-end rotator for a further 5 min, at which point the reaction mixture was loaded onto a PD10 Sephadex° G25 column and eluted with 70 mM HEPES, 2 mM $CaCl_2$, pH 7.0. The protein fraction was collected and dialysed (MWCO 12-14 KDa) against 10 mM MES, 1 mM $CaCl_2$, pH 5.8, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford the glycosylated product m/z (ES) found 27072 calcd. 27078.

B.   SBLS156C mutant (24 mg, 0.89 µmol) was dissolved in aqueous buffer solution (2.4 mL, 70 mM HEPES, 2 mM $CaCl_2$, pH 6.9). 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl phenylthiosulfonate (50mg, 0.1 mmol) was dissolved in water/acetonitrile (1.0 mL, 1/1 ratio). The sugar solution (50 µL) was added to the protein solution and placed on an end-over-end rotator. After 25 min, the absence of free thiol was shown by Ellman's analysis, at which point another portion of sugar solution (50 µl) was added. The reaction was placed on an end-over-end rotator for a further 5 min, at which point the reaction mixture was loaded onto a PD10 Sephadex° G25 column and eluted with 70 mM HEPES, 2 mM $CaCl_2$ pH 7.0. The protein fraction was collected and dialysed (MWCO 12-14 KDa)

against 10 mM MES, 1 mM CaCl$_2$, pH 5.8, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford the glycosylated product m/z (ES) found 27072 calcd. 27078.

C.   SBLS156C mutant (10 mg, 0.37 µmol) was dissolved in degassed aqueous buffer solution (1 mL, 70 mM CHES, 5mM MES, 2 mM CaCl$_2$, pH 9.5). 2,3,6-Tri-O-acetyl-4-O-(2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-O-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranosyl phenylthiosulfonate (30mg, 0.03 mmol) was dissolved in acetonitrile (150 µL). The sugar solution (75 µL) was added to the protein solution and placed on an end-over-end rotator. After 30 min, the absence of free thiol was shown by Ellman's analysis, at which point the reaction mixture was loaded onto a PD10 Sephadex® G25 column and eluted with 70 mM HEPES, 2 mM CaCl$_2$ pH 7.0. The protein fraction was collected and dialysed (MWCO 12-14 KDa) against 10 mM MES, 1 mM CaCl$_2$, pH 5.8, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford the glycosylated product m/z (ES) found 27654 calcd. 27653.

D.   BSA (10 mg, 0.14 µmol) was dissolved in aqueous buffer solution (1 mL, 50 mM Tris, pH 7.7). 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl phenylthiosulfonate (10mg, 0.02 mmol) was dissolved in water/acetonitrile (1.0 mL, 8/2 ratio). The sugar solution (150 µl) was added to the protein solution and placed on an end-over-end rotator. After 30 min, the absence of free thiol was shown by Ellman's analysis, at which point the reaction mixture was loaded onto a PD10 Sephadex® G25 column and eluted with 70 mM HEPES, 2 mM CaCl$_2$ pH 7.0. The protein fraction was collected and dialysed (MWCO 12-14 KDa) against pure water, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford the glycosylated product; m/z (ES) found 66798 calcd. 66794.

E.   BSA (10 mg, 0.14 µmol) was dissolved in aqueous buffer solution (1 mL, 50 mM Tris, pH 7.7). 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl phenylthiosulfonate (25mg, 0.05 mmol) was dissolved in acetonitrile (0.5 mL). The sugar solution (75 µL) was added to the protein solution and placed on an end-over-end rotator. After 30 min, the absence of free thiol was shown by Ellman's analysis, at which point the reaction mixture was loaded onto a PD10 Sephadex® G25 column and eluted with 70 mM HEPES, 2 mM CaCl$_2$ pH 7.0. The protein fraction was collected and dialysed (MWCO 12-14 KDa) against pure water, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford the glycosylated product m/z (ES) found 66792 calcd. 66794.

Example 32: Protein glycosylation procedures using selenenylsulfide reagents

A.   SBLS156C mutant (5 mg) was dissolved in degassed aqueous buffer solution (1 mL, 70 mM CHES, 5 mM MES, 2 mM CaCl$_2$, pH 9.5). Phenyl 2,3,4,6-tetra-O-acetyl-β-D-selenenylsulfide glucopyranoside (10 mg, 0.02 mmol) was dissolved in acetonitrile (500 µl). The sugar solution (500 µl) was added to the protein solution and placed on an end-over-end rotator. After 1 h, the absence of free thiol was shown by Ellman's analysis, at which point the reaction mixture was loaded onto a PD10 Sephadex® G25 column and eluted with 70 mM HEPES, 2 mM CaCl$_2$ pH 7.0. The protein fraction was collected and dialysed (MWCO 12-14 KDa) against water, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford AcGlcSBLS126C m/z (ES) found 27072 calcd. 27078.

B.   SBLS156C mutant (5 mg) was dissolved in degassed aqueous buffer solution (1 mL, 70 mM CHES, 5 mM MES, 2 mM CaCl$_2$, pH 9.5). Phenyl 2,3,4,6-tetra-O-acetyl-β-D-selenenylsulfide glucopyranoside (10 mg, 0.02 mmol) was

dissolved in acetonitrile (800 μl). The sugar solution
(800 μl) was added to the protein solution and placed on
an end-over-end rotator. After 1 h, the absence of free
thiol was shown by Ellman's analysis, at which point the
reaction mixture was loaded onto a PD10 Sephadex® G25
column and eluted with 70 mM HEPES, 2 mM CaCl₂ pH 7.0.
The protein fraction was collected and dialysed (MWCO
12-14 KDa) against water, (1 x 4L for 1 h, 2 x 2L for
30 min), to afford AcGlcSBLS126C m/z (ES) found 66792
calcd. 66794.

C.   SBLS156C mutant (5 mg) was dissolved in degassed
aqueous buffer solution (1 mL, 70 mM CHES, 5 mM MES,
2 mM CaCl₂, pH 9.5). Phenyl 2,3,4,6-tetra-O-acetyl-β-D-
selenenylsulfide galactopyranoside (10 mg, 0.02 mmol)
was dissolved in acetonitrile (500 μl). The sugar
solution (500 μl) was added to the protein solution and
placed on an end-over-end rotator. After 1 h, the
absence of free thiol was shown by Ellman's analysis, at
which point the reaction mixture was loaded onto a PD10
Sephadex® G25 column and eluted with 70 mM HEPES,
2 mM CaCl₂ pH 7.0. The protein fraction was collected and
dialysed (MWCO 12-14 KDa) against water, (1 x 4L for
1 h, 2 x 2L for 30 min), to afford AcGlcSBLS126C m/z
(ES) found 27075 calcd. 27078.

D.   SBLS156C mutant (10 mg) was dissolved in degassed
aqueous buffer solution (1 mL, 70 mM CHES, 5 mM MES,
2 mM CaCl₂, pH 9.5). Phenyl-1-selenenylsulfide-β-D-
glucopyranoside (15 mg, 0.02 mmol) was dissolved in
water/acetonitrile (0.8 mL, 1/1 ratio). The sugar
solution (500 μl) was added to the protein solution and
placed on an end-over-end rotator. After 30 min, the
absence of free thiol was shown by Ellman's analysis,
the reaction was placed on an end-over-end rotator for a
further 30 min, at which point the reaction mixture was
loaded onto a PD10 Sephadex® G25 column and eluted with
70 mM HEPES, 2 mM CaCl₂ pH 7.0. The protein fraction was

collected and dialysed (MWCO 12-14 KDa) against water,
(1 x 4L for 1 h, 2 x 2L for 30 min), to afford
AcGlcSBLS126C m/z (ES) found 27072 calcd. 26911.

E.   SBLS156C mutant (5 mg) was dissolved in degassed
aqueous buffer solution (1 mL, 70 mM CHES, 5 mM MES,
2 mM $CaCl_2$, pH 9.5). Phenyl 2,3,4,6-tetra-O-acetyl-β-D-
selenenylsulfide glucopyranoside (6 mg, 0.02 mmol) was
dissolved in water/acetonitrile (0.7 mL, 2/5 ratio). The
sugar solution (700 µl) was added to the protein
solution and placed on an end-over-end rotator. After 1
h, the absence of free thiol was shown by Ellman's
analysis, at which point the reaction mixture was loaded
onto a PD10 Sephadex® G25 column and eluted with 70 mM
HEPES, 2 mM $CaCl_2$ pH 7.0. The protein fraction was
collected and dialysed (MWCO 12-14 KDa) against water,
(1 x 4L for 1 h, 2 x 2L for 30 min), to afford
AcGlcSBLS126C m/z (ES) found 66792 calcd. 66794.

F.   SBLS156C mutant (5 mg) was dissolved in degassed
aqueous buffer solution (2.4 mL, 70 mM HEPES, 2 mM $CaCl_2$,
pH 6.9). Phenyl -2-acetylamino-2-deoxy-1-
selenenylsulfide-β-D-glucopyranoside (5 mg, 0.01 mmol)
was dissolved in acetonitrile (200 µL, 1/1 ratio). The
sugar solution (100 µl) was added to the protein
solution and placed on an end-over-end rotator. After 30
min, the absence of free thiol was shown by Ellman's
analysis, at which point another portion of sugar
solution (100 µl) was added. The reaction was placed on
an end-over-end rotator for a further 30 min, at which
point the reaction mixture was loaded onto a PD10
Sephadex® G25 column and eluted with 70 mM HEPES,
2 mM $CaCl_2$ pH 7.0. The protein fraction was collected and
dialysed (MWCO 12-14 KDa) against 10 mM MES, 1 mM $CaCl_2$,
pH 5.8, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford
HOGlcNAcSBLS156C m/z (ES) found 26950 calcd. 26950.

F.   SBLS156C mutant (5 mg) was dissolved in degassed aqueous buffer solution (1 mL, 70 mM CHES, 5 mM MES, 2 mM CaCl$_2$, pH 9.5). Phenyl 3,4,6-tri-O-acetyl-2-acetylamino-2-deoxy-1-selenenylsulfide-β-D-glucopyranoside (10 mg, 0.02 mmol) was dissolved in acetonitrile (500 μl). The sugar solution (500 μl) was added to the protein solution and placed on an end-over-end rotator. After 1 h, the absence of free thiol was shown by Ellman's analysis, at which point the reaction mixture was loaded onto a PD10 Sephadex® G25 column and eluted with 70 mM HEPES, 2 mM CaCl$_2$ pH 7.0. The protein fraction was 0collected and dialysed (MWCO 12-14 KDa) against water, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford AcGlcNAcSBLS126C m/z (ES) found 27074 calcd. 27078.

Summary of glycosylation reactions utilising selenenyl sulphide reagents.

Reagent	Synthesis
Glc(Ac)$_4$SSePh	93%
Gal(Ac)$_4$SSePh	95%
Glc(Ac)$_3$NAcSSePh	73%
GlcSSePh	57%
GalSSePh	27%
GlcNAcSSePh	70%

Reagent	EtSH	BocCysThrOMe	SBLS156C	BSA
Glc(Ac)$_4$SSePh	82%	51%	Quant.	Quant.
Gal(Ac)$_4$SSePh	82%	93%	Quant.	
Glc(Ac)$_3$NAcSSePh	93%		Quant.	
GlcSSePh		84%	Quant.	Quant.
GalSSePh				
GlcNAcSSePh			Quant.	

Example 33: Comparison of compounds of formula I with glyco-MTS reagents

In Tables 1 and 2, MTS denotes CH_3-SO_2-S-, and PTS denotes $Ph-SO_2-S-$.

Table 1: Preparation

Glycosylating Reagent	Preparation[1]	
	Total Yield (%)	Steps
Glc(Ac)$_4\beta$-MTS	46[2]	3
Glc(Ac)$_4\beta$-PTS	64	3
Glc(Bn)$_4\beta$-MTS	43[3]	5
Glc(Bn)$_4\beta$-PTS	67	5
Gal(Ac)$_4\beta$-MTS	47	3
Gal(Ac)$_4\beta$-PTS	65	3
Glc(Ac)$_4\alpha$(1,4)Glc(Ac)$_3\alpha$(1,4)Glc(Ac)$_3\beta$-PTS	60	3

1. from the corresponding parent carbohydrate D-glucose (Glc), D-galactose (Gal) or Glcα(1,4)Glcα(1,4)Glc

2. Taken from B.G. Davis, R.C. Lloyd and J.B. Jones, *J. Org. Chem.*, 1998, *63*, 9614, and B.G. Davis, M.A.T. Maughan, M.P. Green, A. Ullman and J.B. Jones, *Tetrahedron Asymmetry*, 2000, *11*, 245.

3.

As shown in Table 1, the glyco-PTS reagents according to the invention were synthesised in superior yields to the corresponding glyco-MTS reagents. Moreover, the costs of the starting materials for synthesis of the glyco-PTS

reagents was approximately ten fold lower than for the corresponding glyco-MTS reagents (at 2003 costs).

In Table 2, SBL-Cys156 is subtilisin *Bacillus lentus* mutant S156C, and BSA-Cys58 is bovine serum albumin.

Table 2. Comparison of glycosylation reactions of glyco-MTS and glyco-PTS reagents.

Glycosylating Reagent	EtSH[1]		Peptide[2]		Protein[3] SBL-Cys156		Protein[3] BSA-Cys58	
	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (min)	Yield (%)	Time (min)
Glc(Ac)$_4\beta$-MTS	96[5]	3	62[5]	5	100[4]	50[4]	–	–
Glc(Ac)$_4\beta$-PTS	82	1	99	5	100	30	100	30
Glc(Bn)$_4\beta$-MTS	78[5]	15	65	4	–	–	–	–
Glc(Bn)$_4\beta$-PTS	95	1.5	82	5	–	–	–	–
Gal(Ac)$_4\beta$-MTS	83	1	–	–	–	–	–	–
Gal(Ac)$_4\beta$-PTS	91	1	95	2	100	30	100	30
Glc(Ac)$_4\alpha$(1,4) Glc(Ac)$_3\alpha$(1,4) Glc(Ac)$_3\beta$-PTS	93	1	74	3	100	30	–	–

1. Et$_3$N, DCM, RT, 1 equivalent (eq.) of thiosulfonate.
2. Et$_3$N, DCM/MeOH (20:1), RT, 1 eq. of thiosulfonate; Peptide [P]-Cys-Ser-OMe, [P] = Ac except for reaction with Glc(Ac)$_4\alpha$(1,4)Glc(Ac)$_3\alpha$(1,4)Glc(Ac)$_3\beta$-PTS where [P] = Boc.

3. 70mM CHES, 5mM MES, 2mM CaCl$_2$ pH 9.5 or 50mM Tris.HCl, pH 7.7, RT, ~30 eq. for glyco-MTS, ~10 eq. for Glc(Ac)$_4\beta$-PTS and Gal(Ac)$_4\beta$-PTS with SBL-Cys156, ~20 eq. for Glc(Ac)$_4\beta$-PTS and Gal(Ac)$_4\beta$-PTS with BSA-Cys58, ~40 eq. for Glc(Ac)$_4\alpha$(1,4)Glc(Ac)$_3\alpha$(1,4)Glc(Ac)$_3\beta$-PTS with SBL-Cys156.

4. Taken from B.G. Davis, R.C. Lloyd and J.B. Jones, *J. Org. Chem.*, 1998, *63*, 9614, and B.G. Davis, M.A.T. Maughan, M.P. Green, A. Ullman and J.B. Jones, *Tetrahedron Asymmetry*, 2000, *11*, 245.

5.

As can be seen from Table 2, the glyco-PTS reagents of the invention generally provided a higher yield in the glycosylation reaction than did the corresponding glyco-MTS compound.

Claims

1. A method of forming a disulfide bond, the method comprising reacting an organic compound comprising at least one thiol group with a reagent of formula I:

$$R—S—X—R^1 \qquad I$$
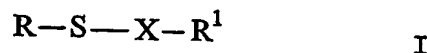
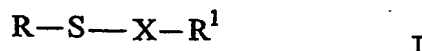wherein:
X denotes SO_2 or Se;
R denotes an organic moiety; and
R^1 denotes an optionally substituted alkyl group, an optionally substituted phenyl group, optionally substituted pyridyl group or an optionally substituted naphthyl group;

with the proviso that when X denotes SO_2 then R^1 does not denote optionally substituted alkyl.

2. A method according to claim 1, wherein the organic compound comprising at least one thiol group is an amino acid, a peptide or a protein.

3. A method of chemically modifying a protein, peptide or amino acid comprising at least one thiol group, the method comprising reacting said protein, peptide or amino acid with a compound of formula I:

$$R—S—X—R^1 \qquad I$$

wherein:
X denotes SO_2 or Se;
R denotes an organic moiety; and
R^1 denotes an optionally substituted alkyl group, an optionally substituted phenyl group, optionally substituted pyridyl group or an optionally substituted naphthyl group;

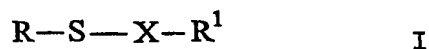with the proviso that when X denotes SO_2 then R^1 does not denote optionally substituted alkyl.

4.   A method according to any one of claims 1 to 3, wherein R is a carbohydrate group.

5.   A method according to any one of claims 1 to 4, wherein R^1 is phenyl.

6.   A method according to any one of claims 1 to 5, wherein X is Se.

7.   A method according to any one of claims 1 to 5, wherein X is SO_2.

8.   A compound of formula I:

$$R—S—X—R^1 \qquad I$$

wherein:
X denotes SO_2 or Se;
R denotes a carbohydrate moiety; and
R^1 denotes an optionally substituted alkyl group, an optionally substituted phenyl group, optionally substituted pyridyl group or an optionally substituted naphthyl group;
with the proviso that when X denotes SO_2, then R^1 does not denote optionally substituted alkyl.

9.   A compound according to claim 8 wherein R^1 is phenyl.

10.   A compound according to claim 8 or claim 9, wherein X is Se.

11.   A compound according to claim 8 or claim 9, wherein X is SO_2.

12. A method for preparing a compound of formula I as defined in claim 11, said method comprising reacting a compound of formula II:

$$M(SSO_2R^1)_k \qquad II$$

wherein:

M denotes a metal, for example Li, Na, K, Ca, Cs, Zn, Mg, or Al; and
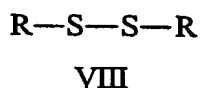
k denotes 1, 2 or 3;

with a compound of formula III:

$$R\text{—}L \qquad III$$

wherein:

L denotes a leaving group.

13. A method for preparing a compound of formula I as defined in claim 11, said method comprising reacting a disulfide compound of formula VIII:

$$R\text{—}S\text{—}S\text{—}R$$
$$VIII$$

with a sulfinite anion of formula $R^1SO_2^-$ in the presence of silver ions.

14. A method for preparing a compound of formula I as defined in claim 10, said method comprising reacting a compound of formula V:

$$R\text{—}SH \qquad V$$

with a compound of formula VI:

$$R^1SeL^2 \qquad VI$$

wherein L^2 denotes Br, Cl, CN, or I.

15. Use of a compound of formula I as defined in any of claims 1 to 7, in disulphide bond formation.

16. Use of a compound of formula I as defined in any of claims 1 to 7, for modifying a protein, a peptide or an amino acid comprising at least one thiol group.

17. Use of a compound of formula I as defined in any of claims 8 to 11, for glycosylating a protein, a peptide or an amino acid comprising at least one thiol group.